2020-06

Effectiveness of biological control and socioeconomic impacts of the invasive parthenium hysterophorus in Arusha, Tanzania

Kanagwa, Warda

NM-AIST

EFFECTIVENESS OF BIOLOGICAL CONTROL AND SOCIO-ECONOMIC IMPACTS OF THE INVASIVE *Parthenium hysterophorus* IN ARUSHA, TANZANIA

Warda Kanagwa

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

June, 2020

ABSTRACT

This study assessed the methods which are likely to be biologically and socio-economically effective to control the invasive species *P. hysterophorus* in Arusha Tanzania. A variety of methods have been found ineffective in long-term control of *P. hysterophorus*. Thus, an effective integrated approach needs to be identified. In addition, perception and socio-economic consequences about *P. hysterophorus* by farmers and pastoralists are still poorly understood. The study assessed the effect of a newly implemented bio-control agent, *Zygogramma bicolorata*, in 4 m^2 field plots. Further a novel approach of controlling *P. hysterophorus* was tested by using herbicidal extracts of the naturalized plants *Dovyalis caffra* and *Cassia auriculata* and compared it with 2,4-D. Finally, 123 farmers and 128 pastoralists in Mbuguni, Olasiti, Murieti and Sepeko wards were interviewed. It resulted that *Z. bicolorata,* particularly at highest population densities, is capable of reducing height of *P. hysterophorus* by 87% and biomass by 91%. There was a significant reduction (50%) in the measured parameters after application of low concentration of extracts of *C. auriculata* leaves and bark and *D. caffra* leaves and fruits (DcL and DcF). Invasion of farm fields by *P. hysterophorus* leads to yield reduction by half according to 21% of farmers, and 46% of farmers claimed that their income was negatively affected. Therefore, this study suggests the use of environmentally friendly bio-herbicides since it can foster *P. hysterophorus* control and emphasize that this method should be integrated with *Z. bicolorata* in the infestation areas for long-term suppression of *P. hysterophorus*.

DECLARATION

I, Warda Kanagwa do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Warda Kanagwa Date: 2nd June 2020

Signature

The above declaration is confirmed

Prof. Anna C. Treydte Date: 2nd June 2020

(Principal Supervisor) Signature

Dr. John Bukombe ……………………. Date………………...

(Co-supervisor) Signature

CERTIFICATION

The undersigned certify that they have read and hereby recommend the dissertation entitled "Effectiveness of biological control and socioeconomic impacts of the invasive *Parthenium hysterophorus* in Arusha, Tanzania" as a fulfilment of the requirement for the degree Master of Life Sciences at the Nelson Mandela African Institution of Science and Technology.

Prof. Anna C. Treydte		Date: 2nd June 2020
(Principal Supervisor)	Signature	

Dr. John Bukombe	…………………….	Date………………...
(Co-supervisor)	Signature	

ACKNOWLEDGEMENT

This work would never been accomplished without the blessing and power of Almighty God, and generous support and kind assistance of many dedicated individuals and not all of whom can be mentioned. I offer my profound gratitude to all. Am extremely grateful to the financial support from Center for Research, Agricultural advancement, Teaching Excellence and Sustainability (CREATES) at The Nelson Mandela African Institute of Science and Technology for the scholarship and Rufford Small Grants for Nature Conservation for funding my research.

My sincere thanks for intellectual debt are extended to Prof. Anna C. Treydte and Dr. John Bukombe under whose supervision of this study was taken. They subtly sharpened my thinking and indicated plausible lines of enquiry, which were likely to bear fruits. More specifically, they are guidance as well as patient and understanding have been invaluable at every stage of this work. I am thankful to the School of Life Sciences and Bioengineering (LiSBE) of NM-AIST for allowing me to use the school laboratory facilities. The advice and technical support from Mr. Damas and Temba, the laboratory specialist is appreciated. Tropical Pesticide and Research Institute (TPRI) is also acknowledged for allowing me to conduct my experiment in their laboratory and field. Also Mr. Ramadhan Kilewa for his great support during the field work and data collection; his support is appreciated.

Special thanks to Mr. Renatus Rwamugira for his encouragement when the project seemed to be hard work. I would also like to thank my friends Neema Mtenga, Margreth A. Makauki, Godiana Philipo, Flora Shonga and Shorastica Mbinile, for their challenging ideas to me in making it the good work.

It will be unfair if I will not acknowledge Mbuguni, Olasiti, Murieti and Sepeko Ward Executive Officers, farmers and pastoralist for their great concern and contribution of making my study very easily during data collection and results dissemination.

I convey my sincere gratitude to my parents Mr and Mrs. Kanagwa, for encouragement and prayers when completion of this work seemed a distance milestone when they required my presence at home. My brother Rashid, for the love and forbearance my sisters Jamillah (Bonge) for her proper grammar and Sharipha (Fifi) for her thoughtful assistance wherever I needed her for the help and when things became more cumbersome.

DEDICATION

This work is firstly dedicated to my God who has given me life and strength to complete this work. In addition, I dedicate this to my family who raised me to this level and able to work hard to make my dreams come true.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
2,4-D	2,4-D Dimethylamine
ANOVA	Analysis of Variance
CaB	*Cassia auriculata* barks
CaL	*Cassia auriculata* leaves
Chl	Chlorophyll content
cm	Centimeter
cm^2	Centimeter square
CRBD	Completely Randomized Block Design
DcF	*Dovyalis caffra* fruits
DcL	*Dovyalis caffra* leaves
df	Degree of freedom
DSMO	Dimethyl sulfoxide
EWRSCS	European Weed Research Society Classification Scale
Fig.	Figure
GIS	Geographical Information system
HIV	Human Immunodeficiency Virus
HSD	Honest significant difference
kg	Kilogram
km	Kilometer
l	Litre
m	Meter
mAb	Monoclonal antibody

mg	Milgram
$Mg.L^{-1}$	Milgram per litre
ml	Mils
mm	Millimeter
N	Population size
nm	Nanometer
NM- AIST	Nelson Mandela African Institution of Science and Technology
ODK	Open Data Kit
PWC	Percentage Weed Control
r^2	R-squared
SPSS	Statistical Package for Social Science
t	T - test
T	Treatment
TPRI	Tropical Pesticide Research Institute
USD	United States Dollar
WAO	Ward Agricultural Officer
WCE	Weed Control Efficiency
WEO	Ward Executive officer
WVO	Ward Veterinary Officer
Z	Mann – Whitney U test
$\chi 2$	Chi square

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Invasive plant species are species that are intentionally or unintentionally introduced from outside of their natural geographic range and are likely to dominate the native species (Cohen *et al.*, 2018). Invasive plants are a threat to biodiversity and pose challenges not only in agriculture, but also in natural resources management since they impact survival and abundance of native species (Foxcroft *et al.*, 2006; Simberloff, 2013). Invasive plants impact ecosystem services, i.e. water, forests, fisheries, climate regulation, crop genetic and pastures of which millions of people depend for their livelihood (Karim *et al.*, 2017). Invasions may cause loss of habitat and may even contribute to human-wildlife conflict since there is more pressure to exploit natural environments for agriculture once current agricultural fields are invaded (Akter & Zuberi, 2009). If left unmanaged, invasive species will also undermine investments in adaptation and mitigation in regards to climate change since many invasive species expand rapidly as the climate warms and outcompete native species (Richardson *et al.*, 2007). Despite efforts to control invasive plant species, they still have a significant impact on biodiversity, crop yields as well as human and animal health. For example, it has been estimated that invasive plant species can reduce forage availability for livestock by 90% and cause a yield loss of 25% in developing countries (Foxcroft *et al.*, 2006).

Parthenium hysterophorus (carrot weed) is an annual, procumbent, herbaceous flowering plant, from the family Asteraceae, an extremely diverse family with a cosmopolitan distribution (Dogra *et al.*, 2011; Mahmoud *et al.*, 2015). *Parthenium hysterophorus* is native to Mexico and the southern United States of America and has become widespread in other parts of the world, including invasions on five continents and numerous islands (Shabbir & Bajwa, 2006; Karim *et al.*, 2017). Adkins and Shabbir (2014) reported that the weed is spread through trade as contaminants of crops and farm machinery. In the early 1950s, *P. hysterophorus* was introduced into Africa and Asia through cereal and seed shipments from America (Evans, 1997). The weed is now widely distributed in many parts of the world including Australia, India, China, the West Indies, Ethiopia, Israel, Taiwan, India and Nepal (Adkins *et al.*, 2018). In Africa, *P. hysterophorus* occurs in several countries including Ethiopia, Somalia, Kenya, Tanzania, South Africa, Swaziland, Zimbabwe, Madagascar and Mozambique (Wabuyele *et al.*, 2014). *Parthenium hysterophorus* is believed to have been introduced into East Africa during the Ethiopia-Somalia war of 1976-1977 (Worku, 2010). *Parthenium hysterophorus* occurs in the northern circuit of Tanzania (Arusha, Kilimanjaro and Manyara Regions) (Kija *et al.*, 2013). The plant is currently spreading to other areas in the country, where it invades cropland, pasture lands, road sides and residential areas (Kilewa & Rashid, 2014).

Parthenium hysterophorus poses serious threats to human health, income, animal health**,** agricultural and pasture production and biodiversity (Seta *et al.*, 2013; Saha *et al.*, 2018). Shrestha *et al.* (2015) found that *P. hysterophorus* is associated with human diseases such as allergic rhinitis, bronchitis, dermatitis, hay fever, allergic eczematous and mental depression. In addition, *P. hysterophorus* causes allergic reactions in cows, buffalo and goats (Ayele *et al.*, 2013). The weed also can reduce the quantity of milk and meat quality (Seta *et al.,* 2013) and poses a substantial problem in rangelands and forestry (Kija *et al.*, 2013). *Parthenium hysterophorus* reduced maize and sorghum production by 40% and 95% in India (Kumar, 2009) and caused a reduction of forage production by 92% in Ethiopia (Adkins & Shabbir, 2014).

To control *P. hysterophorus*, a number of approaches have been used including cultural, physical, and chemical management as well as integrated weed management (Adkins & Shabbir, 2014). Physical methods (uprooting, burning and slashing) are effective in small scale infestations but are not practical and economical in large areas infested with *P. hysterophorus* (Adkins *et al.*, 2018). Furthermore, using herbicides can cause pollution and impact non-targeted organisms (Ngondya *et al.*, 2016). The use of biological methods (use of insects and herbicidal plant extracts) and integrated weed management has recently been emphasized because these methods are sustainable, eco-friendly and cost-effective over large areas (Adkins *et al.*, 2018). A study in Australia found the combined effects of using the biocontrol agent *Epiblema strenuana* along with competition from butterfly pea (*Clitoria ternatea*) and buffel grass (*Cenchrus ciliaris*) reduced *P. hysterophorus* biomass up to 69% (Shabbir *et al.*, 2015).

Various biological control agents have been used successfully against invasive plants. *Pareuchaetes pseudoinsulata* and *Cecidochares connexa* are prioritized as candidate agents in Nigeria and Ghana to suppress *Chromolaena odorata* (Cock & Holloway, 1982). *Epiblema strenuana* has resulted in a significant reduction of *P. hysterophorus* vegetative growth in southern central Queensland (Shabbir *et al.*, 2015). The leaf feeding beetle, *Zygogramma bicolorata* was introduced in South Africa after rigorous research and there was no significant risk to indigenous and economically important flora (Strathie, 2015b). In Tanzania, *Zygogramma bicolorata* has been introduced in some parts of Arusha and Kagera regions by the Tropical Pesticide Research Institute (TPRI). *Zygogramma bicolorata* seems to be an environmentally safe biological agent against *P. hysterophorus* due to its undergoing diapause for more than six months within the soil in the absence *P. hysterophorus* leaves, subsequently emerging with the onset of monsoon when there is plenty of food (Jayanth & Bali, 1995)**.** Similarly, *Z. bicolorata* reduced *P. hysterophorus* height, biomass, flowers and seedbank in India (Shabbir *et al.*, 2016).

Furthermore, recent studies have shown the use of herbicidal plant extracts from both native and naturalized plants may be an effective substitute for synthetic herbicides such as 2,4-D Dimethylamine (Raza *et al.*, 2019). In addition, a study by Omotayo *et al.* (2018) mentioned *Dovyalis caffra* (kei apple) as a good source of bio-herbicide since it has been used to control unwanted crops from areas of cultivation. According to Omotayo *et al.* (2018) if *D. caffra* fruit is soaked in water and allowed to ferment, the liquid produced exhibits herbicidal properties.

Moreover, a study by Khan *et al.* (2013b) has shown that competitive plants can be used as a suppressor to *P. hysterophorus*. *Cassia auriculata* outcompetes *P. hysterophorus* when planted together and reduces its growth and effects its physiology (Khan *et al.*, 2013b). Additionally, *C. auriculata* has been used by traditional healers in Tanzania for medicinal purposes but it is known whether this species' extracts can be used to suppress *P. hysterophorus* in Tanzania.

1.2 Statement of the problem

Attempts to control *P. hysterophorus* include mechanical, cultural, chemical and biological actions (Adkins & Shabbir, 2014). In Tanzania, the common methods used are mechanical (uprooting, slashing and burning) and chemical (mostly the herbicide 2,4-D). Mechanical methods are effective for small scale infestations but are not practical and economical in large areas infested with *P. hysterophorus* (Adkins *et al.*, 2018). Furthermore, mechanical methods are tedious and may cause health impacts to people since allergic reactions and respiratory problems are common in people exposed to the plant (Evans, 1997). Chemical methods are effective; however, these methods are expensive and can cause environmental pollution in addition to affecting non-target organisms (Evans, 1997). Furthermore, *P. hysterophorus* has developed a resistance to 2,4-D making it less effective (Soltys *et al.*, 2013). Therefore, it is practical to consider an integrated approach such as combining a biological control like the leaf-feeding beetle *Z. bicolorata* with herbicidal plant extracts.

Studies by (Ojija *et al.*, 2019; Raza *et al.*, 2019) have shown that herbicidal plant extracts can be used as a part of integrated approach to control *P. hysterophorus*. However, no experiments have been conducted in Tanzania to compare plant extracts of the naturalized plants *Cassia auriculata* and *Dovyalis caffra* with chemical herbicides (2,4-D) for their effectiveness in suppressing *P. hysterophorus*. *Cassia auriculata* contains bioactive compounds like alkaloids, flavonoids, phenols and glyosides (Raj *et al.*, 2012), while *D. caffra* contains polyphenols, a flavonoid that can inhibit plant growth (Omotayo *et al.*, 2018). Both plant species are naturalized in Tanzania, where they are used for medicines (Moshi & Mbwambo, 2002) and fencing materials (Schmelzer, 2008; Chingwaru *et al.*, 2015). In addition, since *D. caffra* has herbicidal properties, especially in their fruits after fermentation (Omotayo *et al.*, 2018), it is also practical to test if this plant can be used to suppress *P. hysterophorus*.

Furthermore, little is known about whether local communities perceive *P. hysterophorus* as a risk to their health and income and whether they are aware of and apply appropriate management techniques in Tanzania. This study assessed community perception and social and economic impacts of *P. hysterophorus* on livelihoods.

1.3 Rationale of the study

Findings from this study will inform institutions responsible for managing *P. hysterophorus* and local communities on the long-term and short-term advantages of using *Z. bicolorata* as a biological control of *P. hysterophorus* and the use of herbicidal extracts. The study will show the role that herbicidal extracts, particularly from *C. auriculata* and *D. caffra*, have on suppressing *P. hysterophorus.* This information will be useful to propose management strategies as a part of an integrated approach for control of the invasive *P. hysterophorus*. It is critical to find novel techniques such as using herbicidal extracts for pest reduction rather than depending on environmentally unfriendly chemicals. Herbicidal extracts are termed natural and an environmentally-friendly technique which may prove to be a unique tool for weed control, increase crop yields, decrease reliance on both synthetic pesticides and improve the ecological environment.

1.4 Objectives of the study

1.4.1 General objective

To identify strategies likely to be biologically and socioeconomically effective to control the invasive species *P. hysterophorus* in Arusha, Tanzania.

1.4.2 Specific objectives

(i) To assess the effectiveness of *Z. bicolorata* in controlling the invasive plant *Parthenium hysterophorus.*

(ii) To compare the effectiveness of extracts from two plant species; *Cassia auriculata* and *Dovyalis caffra* in suppressing *P. hysterophorus* growth in comparison with the chemical herbicide 2,4-D.

(iii) To assess and document the socio-economic impacts of *P. hysterophorus* invasion on the livelihoods of farmers and pastoralists in Arusha.

1.5 Hypothesis

(i) The leaf feeding beetle (*Z. bicolorata*) will be effective in reducing plant height, number of flowers, seedbank and biomass of *P. hysterophorus*.

(ii) *Cassia auriculata* and *Dovyalis caffra* extracts will suppress *P. hysterophorus* growth (root and shoot length, biomass and chlorophyll content), particularly in high concentrations.

(iii) *Parthenium hysterophorus* causes reduced agricultural and livestock productivity which leads to financial losses in communities affected by the invasive plant.

1.6 Significance of the study

This study provides evidence on the effectiveness of an integrated approach to control the invasive plant *P. hysterophorus*. By combining use of a biological control agent, *Z. bicolorata* and herbicidal plant extracts, greater control of the invasive with less detrimental impacts to the environment can be realized than the traditional approach to control of invasive by using chemical herbicides. In addition, these techniques can aid institutions responsible for managing *P. hysterophorus* by informing them on specifics of using these approaches. For instance, the study determined how many beetles are required per hectare and which concentrations of plant extracts are most effective. Furthermore, it is important to determine how local communities, especially farmers and pastoralists, are negatively affected by this invasive species so that to determine appropriate management strategies.

1.7 Delineation of the study

This study assessed the methods which are likely to be biologically and socio-economically effective to control the invasive species *P. hysterophorus* in Arusha Tanzania. It included both screen house experiment, field experiment and questionnaires to farmers and pastoralist. *Zygogramma bicolorata* (a leaf feeding beetle) effectiveness was evaluated under cages condition at Tropical Pesticide Research Institute (TPRI). Naturalized plant extracts materials of *Dovyalis caffra* and *Cassia auriculata* were compared with chemical herbicides 2,4-D under screen house. Lastly farmers and pastoralist were interviewed on social economic effects if *P. hysterophorus* to both people, agriculture and pastoralists.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology, ecology and dispersal mechanism *P. hysterophorus*

Parthenium hysterophorus L commonly referred to as congress weed, ragweed, carrot weed and famine weed is a flowering plant which belongs to the Asteraceae family in the tribe Heliantheae. This is an extremely diverse tribe with a cosmopolitan distribution (Dogra *et al.*, 2011). *Parthenium hysterophorus* is an annual, herbaceous plant with an average height of 1.5 m and heights of up to 2.5 m when growing in good soil and adequate precipitation (Adkins *et al.*, 2018). It is capable of growing and reproducing multiple times per year since it completes its life cycle within four weeks (Akter & Zuberi, 2009). The taproot has both secondary and tertiary roots (Dogra *et al.*, 2011). *Parthenium hysterophorus* leaves are long, dark green, pubescent, rhomboidal and are arranged into narrow lobes on the stem (Hundessa, 2016). The flower of *P. hysterophorus* are creamy-white with a compact head about 3 mm across with five corners each (Seta *et al.*, 2013).

Single *P. hysterophorus* plants are capable of producing up to 100 000 seeds which are very light in weight (Adkins & Shabbir, 2014). The plants are aggressive, adapted to a wide range of soil and climate conditions and produce seeds throughout the year which can lead to four generations of seedlings in one year. However, seedlings emerge better during the wet season so four generations are uncommon (Navie *et al.*, 1998; Seta *et al.*, 2013). *Parthenium hysterophorus* is capable of dispersing seed through livestock and human movements, farm machinery and flooding (Boršić *et al.*, 2018). The seeds are small with short wing-like structures that facilitate dispersal of long distances by water or shorter distances by wind (Tamado *et al.*, 2002).

Parthenium hysterophorus has become a significant weed in more than 90 countries, where it has invaded productive rangelands, posing serious threats to livelihoods and pasture production (Navie *et al.*, 1998; Rwomushana *et al.*, 2019). In Australia, *Parthenium hysterophorus* was first seen in 1955 where it was growing in alkaline and clay-loam soils. However, it tolerates a variety of soil types (Ayele *et al.*, 2013). *Parthenium hysterophorus* has been declared a noxious weed by the governments of Australia, South Africa, Sri Lanka and India, and more recently within the East African region (Guyana & Paraguay, 2014; Boršić *et al.*, 2018).

Therefore, there is a need of understanding its biology, ecology and dispersal mechanism so as to come up with a better management strategy.

2.2 *Parthenium hysterophorus* allelopathic effects

The concept of allelopathy has been defined by Khalaj *et al.* (2013) as the ability of one plant to inhibit the growth and development of other plant by releasing chemicals that impact plant growth. Allelopathic compounds affect germination and growth of neighboring plants by disruption of physiological processes including photosynthesis, respiration, water and hormonal balance (Hassan *et al.*, 2018). Allelopathy was studied in forest ecosystems where it was shown that forestry species had unwanted allelopathic effects on food and fodder crops (Jalali *et al.*, 2013). Allelochemicals are released into the environment by plant organs such as roots, rhizomes, leaves, stems, bark, flowers, fruits and seeds and leaves contain a higher level of allelochemicals (Jalali *et al.*, 2013). Allelopathic interactions are typically negative in character, with positive relations being rare.

Allelochemicals produced by *P. hysterophorus* are sesquiterpene lactones such as parthenin, pseudoguananolides, coronopilin, caffeic acid and flavonoids such asaglycone flavanols (Dogra *et al.*, 2011). These allelochemicals work interdependently and effects include reduced crop and animal production (Dogra *et al.*, 2011). Shabbir and Bajwa (2006) reported that infestations of *P. hysterophorus* can cause a 90% reduction of herbaceous biomass. Natural habitats that have been impacted include grasslands, open woodlands, water courses and bushlands (Seta *et al.*, 2013).

The allelopathic nature of *P. hysterophorus* has changed the physical and chemical characteristics of the soil as well (Wakjira *et al.*, 2009). Once these allelochemicals are dissolved in the soil, they may react with other elements that influence activity of allelochemicals and therefore either amplify or reduce their impact on recipient plants (Msafiri *et al.*, 2013). Wakjira *et al.* (2009) reported that soil pH, soil organic matter, nitrogen, phosphorus and potassium were reduced due to allelochemicals from *P. hysterophorus*.

2.3 Impacts of *P. hysterophorus* on humans

Parthenium hysterophorus may cause allergic reactions (Adamson & Bray, 1999). Health problems can occur either directly from touching the plant or indirectly through contact with airborne particles (Patel, 2011). Up to 20% of people develop asthma, allergic rhinitis**,** bronchitis, dermatitis, hay fever, allergic eczematous or mental depression after exposure to the plant (Evans, 1997; Shrestha *et al.*, 2015). Some of these reactions can be controlled with antihistamine medications, although they are not always effective or obtainable where the weed is found (Adkins & Shabbir, 2014). Patel (2011) correlated exposure to *P. hysterophorus* with health effects like hyperkeratotic papule, diarrhea, breathlessness, chocking and erythematous eruptions. Direct contact can results to positive reaction to mAb-2 and cytokines (Worku, 2010). Skin and pustules and stomach pains may occur (Tanveer *et al.*, 2015). After sun exposure, affected people may develop violaceous papulae and plaque on their exposed parts especially on ears, noses or foreheads (Wakjira *et al.*, 2009). Other health impacts include exacerbating chronic diseases like HIV and tuberculosis (Mcfadyen, 1992).

Due to its ability to flower year round, *P. hysterophorus* provides continuous sugar meals to disease vectors like mosquitoes (Witt, 2010; Nyasembe *et al.*, 2015). This results in increasing longevity of female mosquitos leading to malaria transmission (Stone *et al.,* 2018). In India ill effects associated with *P. hysterophorus* have resulted in mass abandonment of farms due to these direct and indirect impacts that *P. hysterophorus* has on people's health and livelihoods (Witt, 2010).

2.4 Impacts of *P. hysterophorus* on Agriculture

Parthenium hysterophorus negatively impacts a wide range of crops including maize (*Zea mays L*.), sorghum (*Sorghum bicolor L*.) rice (*Oryza sativa L*.) and wheat (*Triticum aestivum L*.) (Shabbir & Bajwa, 2006). After the introduction of *P. hysterophorus* in the 1950s in Australia, the species had spread to almost 17 million ha of grazing pastures by 1994 and to about 60 million ha by 2014 (Shabbir, 2014). Cost of controlling *P. hysterophorus* in Australia is approximately USD 100 million annually (Shabbir *et al.*, 2014). As another example of the economic impacts of *P. hysterophorus*, sunflower (*Helianthus annuus L.*) and sorghum (*S. bicolor)* yields have decreased by 40% and 95% respectively when *P. hysterophorus* invasion was left uncontrolled in India (Tamado *et al.*, 2002). In Ethiopia, the area which is invaded by *P. hysterophorus* has seen decreased forage production of 92% (Tesfu & Emana, 2013). In the Caribbean, 20% of crop losses are due to *P. hysterophorus* while in Kenya, coffee yields have been reduced by 46% by *P. hysterophorus* (Hammerton, 1981). Forty percent of yield reduction in India and 28% in Ethiopia is due to *P. hysterophorus,* which has resulted in food insecurity (Tamado *et al.*, 2002).

2.5 Impacts of *P. hysterophorus* on Livestock

Studies in India, Ethiopia and Australia show that *P. hysterophorus* invasions affect grazing lands by reducing palatable plant production, plant species diversity and community evenness (Thakur, 1994; Kumar, 2009). After invasion of *P. hysterophorus* in India, 35 million ha of land has been affected, resulting in USD 6.7 billion annual investment in management (Kumar (2009). The aggressive colonizing ability of *P. hysterophorus* has resulted in displacement of indigenous plant species which has led to reductions of pasture carrying capacity by as much as 90% (Mcfadyen, 1992; Yadav *et al.*, 2010). In Australia, *P. hysterophorus* has transformed natural vegetation communities including grasslands, open woodlands, river banks and flood plains by inhibiting the growth of native species (Evans, 1997).

Other impacts to livestock includes dermatitis, which causes skin lesions on animals including horses and cattle (Guyana & Paraguay, 2014). Yadav *et al.* (2010) reported that when livestock spend time in the areas dominated with *P. hysterophorus*, they can develop mouth ulcers and subsequent starvation. Acute illness, bitter milk and tainted meat have been observed in animals such as buffalo, goats, and cows (Dhileepan, 2007). Diseases like anorexia, pruritus, alopecia, diarrhea and eye irritation have been reported in animals such as dogs (Kaur *et al.*, 2014). Further health impacts to animals was determined in lab studies: The number of white blood cells was reduced in rats after feeding on *P. hysterophorus* which resulted in weakening of their immune system (Yadav *et al.*, 2010).

2.6 Biological control of *P. hysterophorus*

Biological control (use of plant extracts, microorganisms, insect or fungal pathogens) is a cost-effective, environmentally friendly and practical way of managing *P. hysterophorus* (Adamson & Bray, 1999; Kumar, 2009). In biological control, two main strategies are employed: The classical approach is the introduction of natural enemies from the native range of the invasive; and the augmentative approach in which the populations of natural enemies that are already present are enhanced (Dhileepan, 2007). In the countries where *P. hysterophorus* is very challenging (Australia, India and Sri Lanka), nine insect and two rust biological control agents have been introduced (Gupta & Sharma, 1977; Dhileepan, 2007; Adkins & Shabbir, 2014). In Australia a leaf feeding beetle (*Z. bicolorata*), a stem-galling moth (*Epiblema strenuana*) and a rust (*Puccinia abrupta*) have been shown to be highly efficient in reducing the number of seeds and leaves, especially at the early life history stages of the plant (Dhileepan, 2007; Ray

& Gour, 2012). *Hypothenamus erudistus,* a stem boring scolytid beetle, has been effective in controlling *P. hysterophorus* in India (Kumar, 2009). After introduction of winter rust *P. abrupta* var. in Ethiopia, *P. hysterophorus* growth parameters like height, leaves, branches and biomass were reduced (Taye *et al.*, 2004). Mcfadyen (1992) reported on other biocontrol agents which can be used to control *P. hysterophorus,* including *Listronotus setosipennis, Smicronyx lutulentus, Bucculatrix parthenica* and *Puccinia melampodii*.

Most invasive species control requires the use of multiple biocontrol agents (Dhileepan *et al.*, 2018). However, there have been successes in controlling *P. hysterophorus* using only *Zygogramma bicolorata*. For instance, within three years of the introduction of *Z. bicolorata* in the Bangalore region of India, *P. hysterophorus* infestation was reduced by 85% to 100% (Dhileepan & Strathie, 2009). Similarly, in central Queensland, Australia, *Z. bicolorata* reduced weed density (93%), plant height (65%), biomass (89%), flower production (100%), seedbank (86%) and seedling emergence (90%) (Dhileepan, 2007; Kumar, 2009). The use of *Z. bicolorata* in South Africa has been successful in most of the areas in which it was introduced (Strathie, 2015a). After seeing the successes in South Africa, Tanzania initiated release of this leaf-feeding beetle in March 2017 (Kilewa, personal communication, April 22, 2018). *Z. bicolorata* has been introduced in Arusha and Kagera regions.

In addition to introduction of living biocontrol agents, scientists are exploring the efficacy of plant extracts to control invasive species (Ngondya *et al.*, 2016; Raza *et al.*, 2019). The study by Javaid *et al.* (2010) revealed that flavonoids extracted from mango leaves can reduce germination and growth of *P. hysterophorus*. In addition, metabolites of fungal species may have herbicidal effects on the germination and growth of *P. hysterophorus* (Javaid *et al*., 2010). Similarly, aqueous extracts from *Imperata cylindrical, Desmastachya bipinnata* and *Sorghum halepense* cause mortality of *P. hysterophorus* (Ali & Khan, 2017). Furthermore, scientists in India believe that species such as *Cassia auriculata, Croton bonplandianum, Amaranthus spinosus, Tephrosia purpurea, Hyptis suaveolens, Sida spinosa, Cassia sericea* and *Cassia tora* are capable of reducing *P. hysterophorus* infestations in natural habitats (Khan *et al.*, 2010). *Cassia sericea* reduced *P. hysterophorus* population sizes by 70% and 50% (Khan *et al.*, 2013b). In Northern Tanzania, plant extracts have been used successful; Ngondya *et al.* (2016) found *Desmodium uncinatum* leaf extract reduced *Gutenbergia cordifolia*'s ability to perform photosynthesis and led to stunted growth.

2.7 Mechanical control of *P. hysterophorus*

Uprooting, burning and slashing is effective as a first-line management of *P. hysterophorus*: When undertaken before *P. hysterophorus* flowering, mechanical methods are capable of reducing its population by 75% (Strathie, 2015a). In India, mechanical methods reduced *P. hysterophorus* density (Kaur *et al.*, 2014). A negative aspect of mechanical control may be the high labor requirements. This is especially concerning in a plant such as *P. hysterophorus* since exposure to it may lead to health problems to humans (Adkins & Shabbir, 2014). The cost of managing *P. hysterophorus* using mechanical control method is estimated at USD 2 billion in India (Mcfadyen, 1992), while in the United Arab Emirates the estimated cost is USD 550 million (Dogra *et al.*, 2011). Furthermore, there may be other negatives to using mechanical methods to control *P. hysterophorus*. A study in Australia showed that burning is not an effective method of controlling *P. hysterophorus* since fire requires large quantities of fuel and destroys other economically important plants in the vicinity (Kaur *et al.*, 2014). Also, using mechanical methods after flowering may actually enhance the invasive species since it could aid in seed dispersal (Dhileepan, 2007).

Mulching may also be an effective mechanical control method for *P. hysterophorus* (Khaket *et al.*, 2015*)*. In Sri Lanka, mulching with *Gliricidia sepium* leaves suppressed the growth and development of *P. hysterophorus* and increased yield in tomato farms (Kaur *et al.*, 2014). In addition, mulch may have other benefits to agricultural systems since it conserves moisture, lowers surface temperature, fertilizes the soil, offers protection from soil erosion and improves soil quality.

2.8 Chemical control of *P. hysterophorus*

The use of chemical herbicides, as either pre- or post-emergent treatments, has been effective in controlling *P. hysterophorus* (Adkins & Shabbir, 2014). These chemical have different active ingredients and modes of action (Dhileepan & Strathie, 2009; Adkins & Shabbir, 2014). The herbicides 2,4-D Dimethylamine, glyphosate, paraquat and atrazine are common in *P. hysterophorus* control (Gnanavel & Natarajan, 2013). Chemical herbicides, especially 2,4-D and glyphosate, have been reported to be effective in controlling invasive *P. hysterophorus* in North America and in some parts of Sri Lanka (Wagner *et al.*, 2017).

Application of these chemicals should be when the plants are young at a pre-flowering stage (Adkins & Shabbir, 2014). For the chemical to be effective, it is necessary to monitor the treated area for at least seven years and any plant coming from the seedbank should be sprayed (Adkins & Shabbir, 2014). In wastelands, along roads and non-cropped areas, the use of sodium chloride at 15% to 20% concentration is effective (Gupta & Sharma, 1977). Despite chemical control being the most common method used against *P. hysterophorus,* it is becoming less effective due to the development of resistance. In addition, many negative impacts to the environment have been reported (Mishra & Bhan, 1996).

2.9 Public awareness (outreach program and capacity building)

Successful management of invasive species involves the active participation of local communities (Batish *et al.,* 2004). Basic biology and ecology of the weeds should be taught to farmers, pastoralists, students and the general public so that they can recognize seedlings, removing them at an early growth stage (Adkins *et al.*, 2018). For example, in India, after intensive capacity building to locals on *P. hysterophorus* biology and impacts, people were able to participate in the manual removal of the weed and a 300 m by 11 m, heavily-infested roadside was restored with native species (Batish *et al.*, 2007). However, these manual removal control methods of this weed suffer from several limitations. Proper disposal is necessary after hand pulling. Tamado *et al.* (2002) indicated that hand hoeing before flowering is time consuming and labor intensive. Furthermore, they reported that manual cutting such as slashing or mowing results in rapid regeneration, quick flowering and excess seed production.

Singh *et al.* (2004) described how local communities were trained on the use of chemical methods for the control of *P. hysterophorus*. They found that atrazine plus 2,4-D at 0.5 kg per hectare of each chemical caused 45% mortality of *P. hysterophorus* when applied to young plants. Similarly, Mishra and Bhan (1996) showed how local communities used chloromuron and metasulfuron to effectively control *P. hysterophorus* especially if applied to young plants (<30 cm tall). However, in areas densely covered by *P. hysterophorus,* chemical control is not a viable option because vegetation is so dense that it will not kill the plant (Navie *et al.*, 1996). A further limitation of chemical management methods is that they are environmentally unsafe and costly (Baryakabonaa & Mwine, 2017).

Therefore, alternate methods are needed to control this invasive species. Joshi (1991) found that training local communities on the use of competitive plants offers a potential solution, but studies are scarce. For instance, the leguminous sub-shrub *Cassia uniflora*, is capable of replacing invasive *P. hysterophorus* (Joshi, 1991). Similarly, aqueous extracts of the allelopathic grasses *Dicanthium annulatum*, *Cenchrus pennisiformis*, *Imperata cylindrical* and *Sorghum helepense* suppress germination and growth of *P. hysterophorus* (Anjum *et al.*, 2005; Javaid *et al.*, 2005).

2.10 Integrated management

Integrated management (IM) combines methods to control invasive species. It is considered to be the most effective approach to long-term control of invasive species, including *P. hysterophorus* control (Adkins & Shabbir, 2014; Adkins *et al.*, 2018). Several countries have used integrated pest management as an approach to controlling *P. hysterophorus,* since one method alone has been insufficient for sustainable control (Shabbir *et al.*, 2015). In Australia, the combined effects of using the biocontrol agent *Epiblema strenuana,* along with competition from butterfly pea and buffel grass, reduced *P. hysterophorus* biomass up to 69% (Shabbir *et al.*, 2015). Similarly, in India, integration of community efforts and other land management approaches provided an effective management approach for *P. hysterophorus* weeds and was more effective than using individual approaches (Kohli *et al.*, 2006).

2.11 Characteristics of *Cassia auriculata*

Cassia auriculata L. (Tanner's Cassia), a leguminous tree that grows 3-7 m high, belongs to the sub-family *Caesalpinioideae* (Nille & Reddy, 2015). It is a native of India and Sri Lanka and was introduced and became naturalized into several African countries (Nille & Reddy, 2015). It has been grown as an ornamental in India, Nigeria, Ghana and Tanzania (Ayyanar & Ignacimuthu, 2008). Its roots, seeds, leaves, flowers, seeds and bark have been utilized for medicines by both traditional healers and the pharmaceutical industry (Gupta *et al.*, 2009). In India, this plant treats jaundice, liver disease, inflammation of the eye or conjunctiva, liver damage seen in alcoholics, skin disease, asthma, rheumatism, constipation and urinary tract disorders (Gupta *et al.*, 2009). In Tanzania, *C. auriculata* is used by traditional healers to treat diabetes (hypoglycemia), eye infections, joints, muscle pain and urinary tract disorders (Moshi & Mbwambo, 2002). Khan *et al.* (2013b) reported *C. auriculata* outcompetes *P. hysterophorus* when planted together and reduces its growth and effects its physiology (Khan *et al.*, 2013b).

2.12 Characteristics of *Dovyalis caffra*

Dovyalis caffra (Kei apple), a dioecious, evergreen tree that grows 3-8 m high, belongs to subfamily *Angiosperm* (Copeland *et al.*, 2006). It grows above 1200 m in elevation, prefers deep, well-drained soils, and is drought resistant once established (Geldenhuys, 1992). Its 6 cm diameter fruit is spherical, fleshy and turns from green to yellow-orange and has a velvety surface when mature. The fruit has persistent styles containing about 10-15 seeds each (Lemaire, 1990). The fruit is rich in vitamin C and can be eaten raw or made into jelly and jam, while the leaves are used as fodder. *Dovyalis caffra* also provides valuable forage for bees. *Dovyalis caffra* is planted as a live fence and ornamental tree in Tanzania, Uganda, and Mozambique (Copeland *et al.*, 2006). Furthermore, when the fruits are allowed to ferment, it has been used as a weed killer in agriculture (Copeland *et al.*, 2006; Omotayo *et al.*, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted in Arusha, Tanzania (3°S, 36°E; Fig. 1). It focused on the Arusha urban, Arumeru and Monduli districts, which are highly infested by *P. hysterophorus* (Wabuyele *et al.*, 2014). The area has bimodal rainfall with long rains from February to June and short rains from October to November (Funk *et al.*, 2015). Annual rainfall of Arusha generally ranges between 800 - 1100 mm (Funk *et al.*, 2015). The climate is warm with mean annual temperature of 19.2 °C (Funk *et al.*, 2015). The soils are commonly black cotton, of a silt texture and deep and freely draining; this soil is easily eroded. These soils have a very low bearing strength and hence poor traction, which might favor the spread of *P. hysterophorus*. During the dry season, the surface layers become very powdery and dusty, especially after being loosened by foot traffic, animal hoofs and machinery (Mahugija, 2013). These soils are fertile and thus have a high value for agricultural use (Funk *et al.*, 2015).

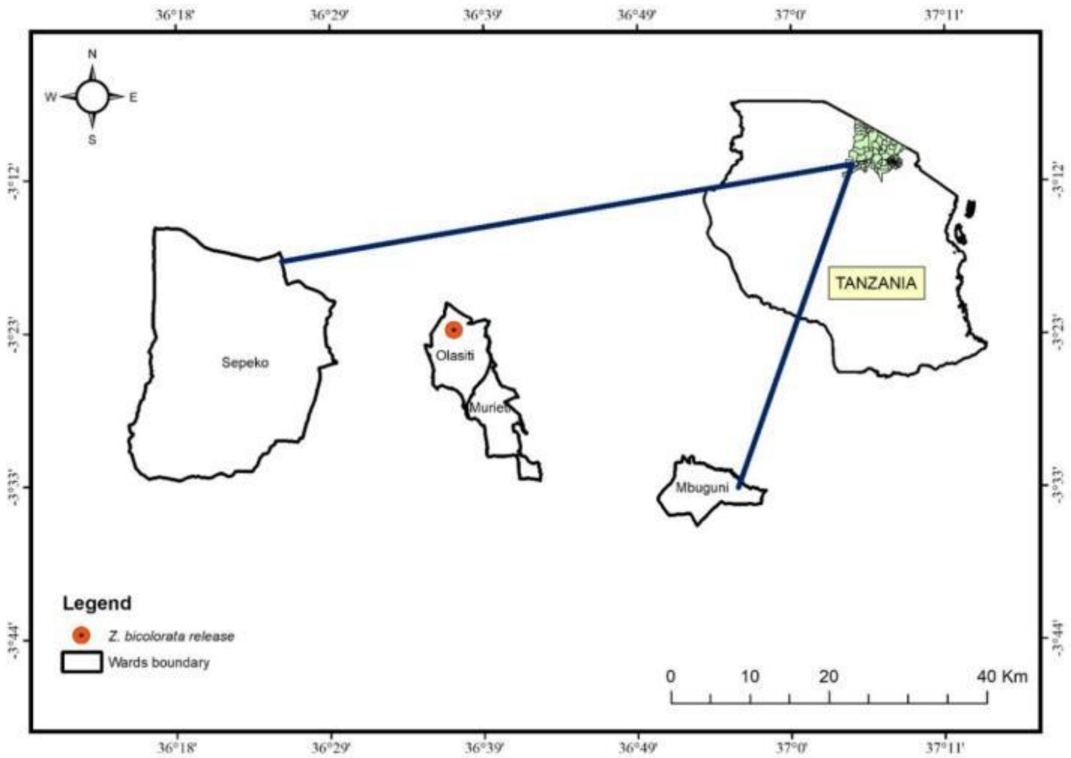

Figure 1: Map of the study area showing the selected wards and areas where *Z. bicolorata* was released

3.2 Effectiveness of *Z. bicolorata* in controlling *P. hysterophorus*

The effectiveness of *Z. bicolorata* as a biocontrol agent of *P. hysterophorus* was evaluated under cage conditions at the Tropical Pesticides Research Institute (TPRI) in Arusha, Tanzania (3°19' 51.35"S, 36°37' 37.602"E) from February to July 2019 (Plate 1). This study was long enough to complete life cycles of both *Z. bicolorata* and *P. hysterophorus*.

Plate 1: Experimental site showing "A" The ground for the experiment, "B" 4 m x 4 m cages at the Tropical Pesticide Research Insititute (TPRI)

3.2.1 *Parthenium hysterophorus* seed collection and nursery establishment

About 30 000 seeds of *P. hysterophorus* were collected in the field from individual, mature plants that grew at least 5 m away from each other in Burka and Ngaramtoni wards in Northern Tanzania during the month of January 2019. Seeds were washed with distilled water, disinfected with 4% NaOCl for 10 minutes, and air dried for four days (Kigel, 2017). Seeds were then germinated in the TPRI nursery.

3.2.2 *Zygogramma bicolorata* collection

Adult *Z. bicolorata* were collected from *P. hysterophorus* plants in Burka ward, about 18 km away from TPRI, where they had been released in a test trial by TPRI in 2017 (Plate 2A). The beetles were collected in April, during the rainy season, when *Z. bicolorata* were emerging from the soil after diapause. Beetles were transported to the TPRI laboratory and mated pairs were identified (Plate 2B).

Plate 2: *Zygogramma bicolorata* preparation. "A" represents areas where the beetle was collectedin the field in April, "B" identification of male and female beetles at the laboratory of TPRI and "C" *Z. bicolorata* release in experimental field plots at TPRI in May 2019

3.2.3 Experimental design

A randomized block design of four treatment levels (0, 10, 20 or 30 beetles with half female and half male) was used in each cage (block) with three replicates (Figure 3C). Cages were 4 m^2 with a distance of 25 cm between each plant and each row within the cage. There was a distance of 1 m between cages (Shabbir *et al.*, 2016). White mesh nets were used to cover each cage to prevent beetles from escaping. On day 14, 25 seedlings of *P. hysterophorus* were transplanted into each cage. Beetles were introduced on day 24 of the experiment. The nine interior plants plus one additional plant were selected in each plot and tagged and measurements were repeated on these plants.

3.2.4 Measurements

During data collection number of leaves eaten and number of flowers were recorded at 14-day intervals. Additionally, plant height of each tagged plant and its total biomass were measured at the end of the experiment **(**day 56). Numbers of beetle **e**ggs, larvae and adults were counted in each plot every fourteen days. On day 56, after the adult plants of *P. hysterophorus* were harvested, the plots were watered every three days to allow any *P. hysterophorus* seeds to germinate. The number of seedlings emerging in the plots was recorded every seven days for an additional 56 days. After each seedling was counted, it was removed.

3.2.4 Statistical analysis

Data were first tested for normality and homogeneity of variance using Shapiro-Wilk and Levine’s test (Shapiro & Wilk, 1965). For the number of leaves eaten and number of flowers produced a mixed model repeated measures analysis was used with week, treatment and week by treatment as fixed effects and block (cages) and individual plants as random effects. For seedbank and number of eggs, larvae and adults a mixed model repeated measures analysis was used with week, treatment and week by treatment as fixed effects and block (cages) as a random effect. For height, fixed effect was treatment and random effect was block (cages). Planned contrasts were used to compare the dependent variables within each week with a one-way analysis of variance. All the data were subjected to JAMOVI 1.2.2 and STATISTICA version 8 Stat Soft Inc. (2007) were used with Tukey’s HSD to control for errors due to multiple comparisons. The level of significance was $\alpha = 0.05$.

3.3 Field survey in the area with and without *Z. bicolorata*

This study compared damage to vegetation by insects in Burka ward in the release area to a control area about five km distant from where *Z. bicolorata* was introduced in 2017 (Plate 3). In both the release and control areas, two line transects was established with ten 3 m x 3 m plots along each transect. In each area a 1 m x 1 m quadrat was located at four places within the 3 m x 3 m plot. In each 1 m x 1 m quadrat, the number of damaged *P. hysterophorus* plants and plant height was recorded along with the numbers of other broad-leaved forbs, grasses and sedges. Data were first tested for normality and homogeneity of variance using Shapiro-Wilk and Levine’s tests (Shapiro & Wilk, 1965). The data were analysed using a two-sample t-test to compare *P. hysterophorus* damage in the areas with and without *Z. bicolorata.*

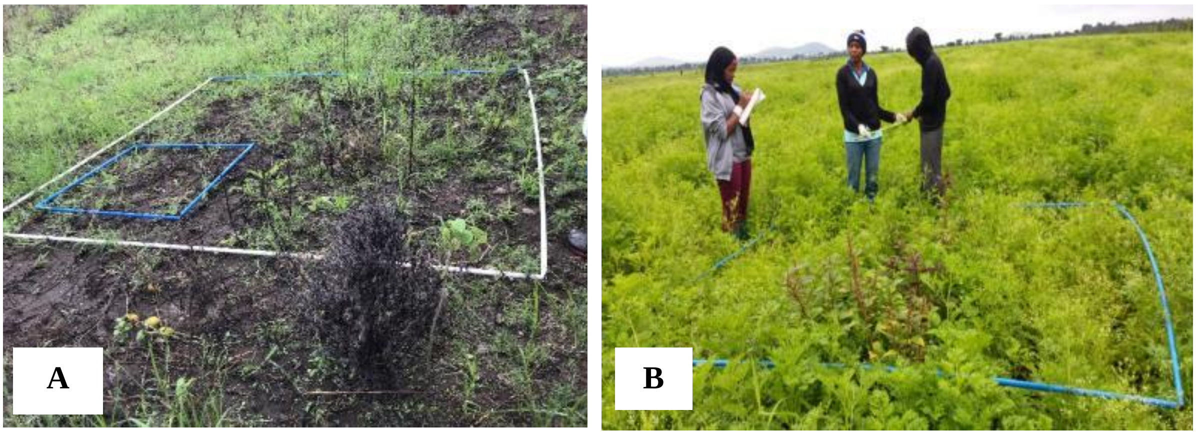

Plate 3: Field experiment showing "A" an area where *Z. bicolorata* was released and "B" an area where it was not released

3.4 Effectiveness of *C. auriculata* and *D. caffra* in suppressing *P. hysterophorus* vegetative growth

3.4.1 Preparation of plant material

Fifteen kg of leaves (CaL) and 10 kg of bark (CaB) of adult *C. auriculata* and 15 kg of *D. caffra* leaves (DcL; Plate 4A) were collected from individuals >10 m away from each other (Raza *et al.*, 2019) in Mbuguni, King'ori, Kikwe and Murieti wards in Arusha region in January 2019. Materials were transported to the Nelson Mandela African Institution of Science and Technology (NM-AIST) laboratory. All samples were washed with distilled water, air dried at room temperature for 21 days and ground (Ngondya *et al.*, 2016). Material was then sieved through ha 25 mm diameter mesh screen to ensure a homogeneous mixture. A mixture of 100 g of leaves or bark was immersed in 1 litre distilled water. After three days, the solution was filtered using Muslin cloth and No. 1 Whatman filter paper (Ojija *et al.*, 2019). These stock solutions of 0.1 g ml^{-1} were then diluted into three concentrations to obtain 30 g l^{-1} (30%), 60 g l^{-1} (60%) and 90 g l^{-1} (90%) (Raza *et al.*, 2019). Extract solutions were stored at 4 ^{o}C prior to application (Ngondya *et al.*, 2016).

Furthermore, extracts from the fruits of *D. caffra* (DcF) were prepared. Fruits were collected from Ngaramtoni, Olasiti and Kikatiti wards and washed with distilled water (Plate 4C). Samples of 500 g (30-33 fruits), 1000 g (63-66 fruits), and 1500 g (90-96 fruits) were soaked in 3 l of distilled water and allowed to ferment for 4 days (Minnaar *et al.*, 2017; Omotayo *et al.*, 2018). Fermented samples were filtered using Muslin cloth and No. 1 Whatman filter paper to obtain concentration of 0.5 g l^{-1}, 1 g l^{-1} and 1.5 g l^{-1} (Plate 4D).

In addition to these natural extracts, a 2,4-D Dimethylamine synthetic herbicide treatment was prepared of the recommended concentration to use for *P. hysterophorus* (Kundu *et al.*, 2018). For the 0.1% solution, 10 ml of the herbicide was diluted in 1 l distilled water.

Plate 4: Experimental materials "A" leaves of *D. caffra*, "B"stock solution dilution, "C" caffra fruits and "D" fruits of *D. caffra* after fermentation process

3.4.2 Experimental design

Completely Randomized Block Design (CRBD) with four replicates was used to test whether different concentrations of *C. auriculata* or *D. caffra* extracts can suppress *P. hysterophorus* as well or better than the herbicide 2,4-D. Approximately 1500 seeds of *P. hysterophorus* were collected from individuals that grew at least 5 m distance from each other at Burka and Ngaramtoni wards in January 2019. Seeds were washed with distilled water, disinfected with 4% NaOCl for 10 minutes (Kigel, 2017), air dried for four days and then germinated in screen house at the Tropical Pesticides Research Institute (TPRI; $3^{\circ}19'$ 51.35"S, $36^{\circ}37'$ 37.602"E). Twenty plants of *P. hysterophorus* were planted into 57 pots (780 cm^2 surface area) for a total of 1140 plants. Each pot received a separate treatment. After 10 days, when *P. hysterophorus* had obtained shoot lengths of approximately 7 cm, plants were sprayed with T0 = 0%, T1= 30%, T2 = 60% and T3 = 90% of CaL, CaB, DcL, 0.5 g l^{-1}, 1 g l^{-1} and 1.5 g $l^{-1}$of DcF or 10 ml l^{-1} of 2,4-D (Table 1). Foliar spray was applied using a 2 l hand pressure sprayer in the evening to retain active ingredients (Baryakabonaa & Mwine, 2017). Control pots were sprayed with distilled water. Treatments were applied three times at an interval of three days (Day 7, Day 10 and Day 13).Tap water was added as necessary to all plants to ensure no plants suffered from drought stress (Ngondya *et al.*, 2016).

Table 1: Treatment used for this experiment

Treatments	Dosage
Control	0
CaL	30%
CaL	60%
CaL	90%
CaB	30%
CaB	60%
CaB	90%
DcL	30%
DcL	60%
DcL	90%
DcF	0.5 g l^{-1}
DcF	1 g l^{-1}
DcF	1.5 g l^{-1}
2, 4-D (Standard)	10 ml l^{-1}

3.4.3 Parameters measured

Following Hiscox and Israelstam (1979), *P. hysterophorus* leaves were analyzed for total chlorophyll content after 28 days. Leaf samples of 70 mg of *P. hysterophorus* were placed in a falcon tube containing 7 ml of Dimethyl Sulfoxide (DMSO). Tubes were incubated for 24 h at 65 °C, and then diluted with 3 ml of DMSO. Absorbance was measured at 645 nm and 663 nm using a spectrophotometer. Total Chlorophyll content levels (Chl) was calculated according to Arnon (1949): Chl = 20.2 D_{645} + 8.02 D_{663}, where D_{645} and D_{663} are absorbance readings at 645 nm and 663 nm respectively. In addition, dry and fresh biomass, plant height (root and shoot lengths) and percentage weed control was measured after 28 days. Dry matter was measured after drying samples at 65 ˚C for 48 h.

Percentage weed control (PWC) per treatment was estimated according to the formula used by Carson (1979);

$$\text{PWC} = 100 - \left(\frac{Number\ of\ weeds\ per\ treatment}{Number\ of\ weeds\ per\ weed\ control}\right) \times 100$$

Percentage activity of plant extracts (CaL, CaB, DcL and DcF) was classified by using the European Weed Research Society (EWRS) scale of 1-9 where 1 indicates complete weed control and 9 indicates no weed control (Table 2). One-way ANOVA was performed to test for a significant difference in PWC across treatment.

Table 2: European Weed Research Society (EWRS) classification scale representing the percentage limit of acceptabilty

EWRS scale	% Activity	
1	100	
2	98-99.99	
3	95-97.90	
4	90-94.90	
5	**82-89.9** ←	Limit of acceptability
6	70-81.9	
7	55-69.9	
8	30-54.9	
9	0-29.9	

3.4.4 Statistical analysis

Data were first tested for homogeneity of variance using Levene's test and normality using Shapiro-Wilk test (Shapiro & Wilk, 1965). The independent variable was chemical concentration while dependent variables were growth parameters (root length, shoot length, fresh biomass, dry biomass and total chlorophyll content). Effectiveness of the treatments CaL, CaB, DcL, DcF and 2,4-D effects were tested using One-way ANOVA on untransformed data since all data were normally distributed. Significant differences in treatment means were determine during Tukey's HSD to control for error due to multiple comparisons using STATISTICA version 8 Stat Soft Inc. (2007) at $\alpha = 0.05$ level of significance. Origin version 9.0 SR1 (2013) was used to make box plots.

3.5 Socio-economic impacts of *P. hysterophorus* invasion on livelihoods of farmers and pastoralists

3.5.1 Study design and sampling technique

Olasiti, Murieti, Mbuguni and Sepeko wards were selected to determine socio-economic impacts of *P. hysterophorus* after a reconnaissance survey revealed high levels of *P. hysterophorus* infestation. Numbers of households to sample were determined following Badjie *et al.* (2019). A total of 123 farmers and 128 pastoralists were selected. To ensure a wide range of coverage and relative independence, each interviewee had lived in the area for at least 10 years and their houses were at least 200 m away from each other (Fig. 2). Data were collected from February to May 2019 through household surveys, key informant interviews with Ward Executive Officers (WEO), Ward Agricultural Officers (WAO) and Ward Veterinary Officers (WVO), as well as through participatory field observation (Plate 5). Semi-structured questionnaires were composed of both open-ended and closed questions. Open Data Kit (ODK data collection) was employed to pretest the survey design for validity and comprehensiveness (Mussa *et al.*, 2017). In the areas where local communities did not understand Swahili, such as Sepeko ward, local translators were employed. Impacts of *P. hysterophorus* on farmers and pastoralists were assessed together with *P. hysterophorus* level of infestation interviewees perceived in their local landscape and the methods employed by them or other community members in controlling this aggressive weed. The study also assessed the knowledge about current biocontrol methods.

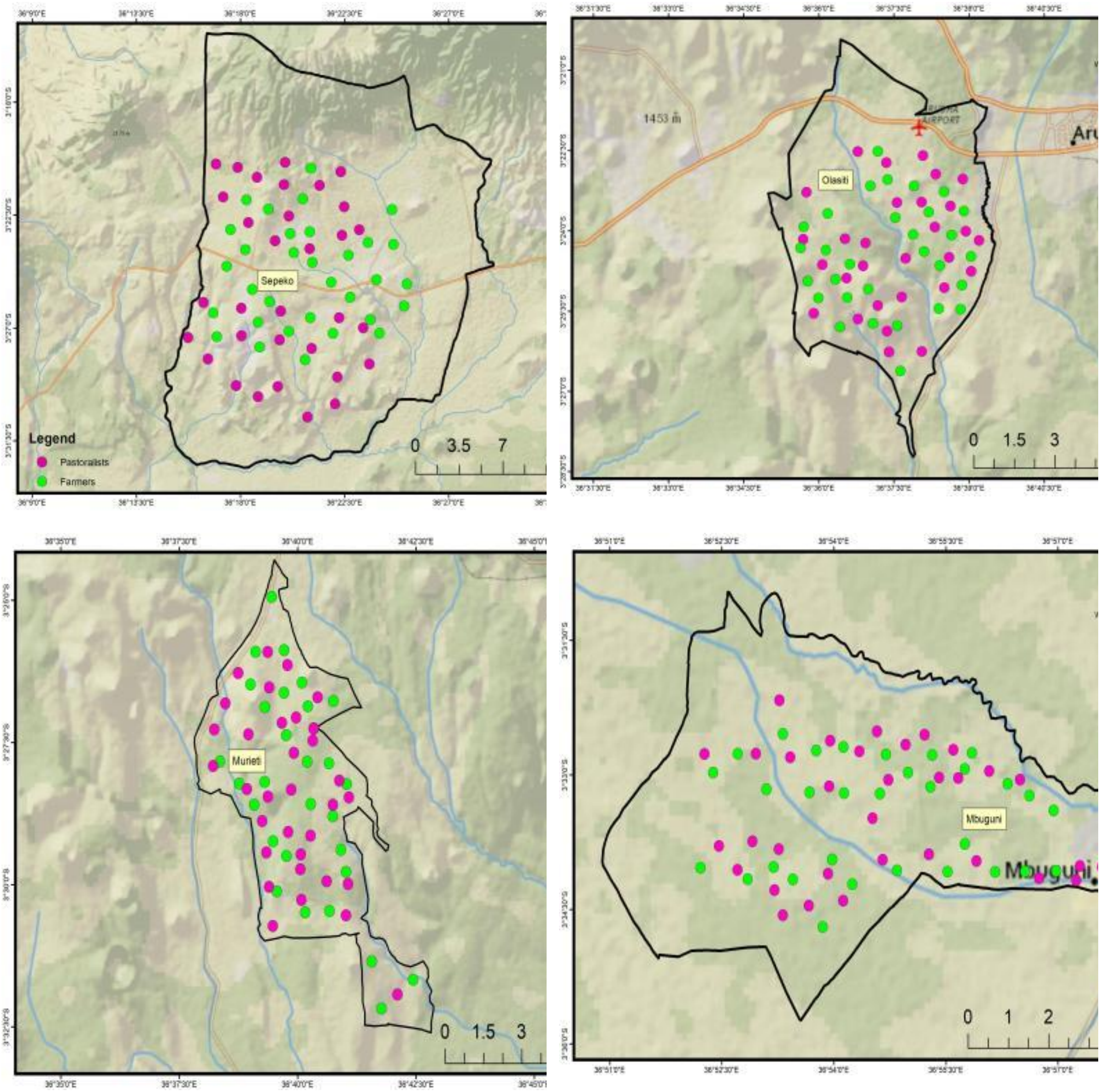

Figure 2: Map of study areas showing how farmer and pastoralist households that were interviewed were distributed within the Arusha, Tanzania

Plate 5: The researcher interviewing some of the household members

3.5.2 Statistical analysis

Data were tested for normality using Shapiro-Wilk and Kolmogorov-Smirnov tests. Data analysis was performed in Statistical Package for Social Sciences (SPSS v.20) at $\alpha = 0.05$ level of significance. *Parthenium hysterophorus* knowledge (how to control the weed and its impacts to both farmers and pastoralists) was compared across wards using the non-parametric Mann–Whitney U-tests since data did not meet the assumptions of normality for parametric analysis. In addition, correlation analyses were performed to compare the relationship between level of education and knowledge about *P. hysterophorus* and its management. Chi square tests were performed for categorical variables. Graphs were plotted using Origin version 9.0 SR1(2013). The distribution of interviewed households in the study area was mapped using Arc GIS (Arc Map version 10.6).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Effects of *Z. bicolorata* on *P. hysterophorus* vegetative growth

The level of defoliation of *P. hysterophorus* increased with time and the effects were seen in all treatments of *Z. bicolorata* with higher numbers of beetles leading to higher levels of defoliation (Fig. 3). There was a strong significant difference among week, treatment and week by treatment (Table 3). The 30 beetle treatment always showed the highest number of leaves eaten, beginning with approximately 10 leaves eaten on day 14 and exceeding 40 leaves eaten on average by day 56 while there were never any leaves eaten in the control. The percentage of *P. hysterophorus* leaves eaten by *Z. bicolorata* increased rapidly from 14 to 28 days by 100 to 115% (Fig. 3). There was a significant difference across treatments at 14 and 28 days after the *Z. bicolorata* release ($F_{3,8} = 58.8, p < 0.001$ and $F_{3,8} = 39.3, p < 0.001$ respectively). In the subsequent periods (28 to 42 days after beetle release), the percentage of leaves eaten increased by 107% (10 beetles), 83% (20 beetles) and 75% (30 beetles). These differed significantly across treatments at 42 days after the *Z. bicolorata* release ($F_{3,8} = 98.6, p < 0.001$). Similarly, from day 42 to 56, there was an increase of leaves eaten by 62% (10 beetles), 26% (20 beetles) and 3% (30 beetles), showing a significant difference across treatments at 56 days after *Z. bicolorata* release at $F_{3,8} = 57.2, p < 0.001$ (Fig. 3).

Number of flowers increased significantly early in the experiment, especially in the control and 10 beetle treatments, reaching a maximum of over 60 flowers per plant subsequently decreasing. However, the 20 and 30 beetle treatments had only a maximum of about 20 flowers per treatment. There was a strong significant difference among week, treatment and week by treatment (Table 3). Early in the experiment (14 to 28 days), there was an increase in the number of flowers by 386% (0 beetles), 194% (10 beetles), 109% (20 beetles) and 102% (30 beetles). The highest number of flowers was recorded after 28 days of the experiment with a significant difference among treatments ($F_{3,8} = 36.1, p < 0.001$). Later in the experiment (42 days after beetle release), the number of flowers was significantly lower than that of 28 days by 7% (0 beetles), 17% (10 beetles), 39% (20 beetles) and 41% (30 beetles) ($F_{3,8} = 57.1, p < 0.001$). From day 42 to 56, the number of flowers reduced even further by 28% (0 beetles), 41% (10 beetles), 62% (20 beetles) and 88% (30 beetles), which was significant at $F_{3,8}= 35.9$, $p= 0.001$ (Fig. 4).

Table 3: Repeated measures analysis showing results for each mixed model of number of leaves eaten and number of flowers including week, treatment and week by treatment as fixed effects, while cages and plants were random effects

	Number of leaves eaten		Number of flowers	
	F statistics	***p***	***F statistics***	***p***
Week	$F_{3,32} = 86.8$	< 0.001	$F_{3,30} = 41.4$	< 0.001
Treatment	$F_{3,35} = 227.2$	< 0.001	$F_{3,32} = 85.1$	< 0.001
Week*treatment	$F_{3,36} = 15.4$	< 0.001	$F_{9,32} = 11$	< 0.001

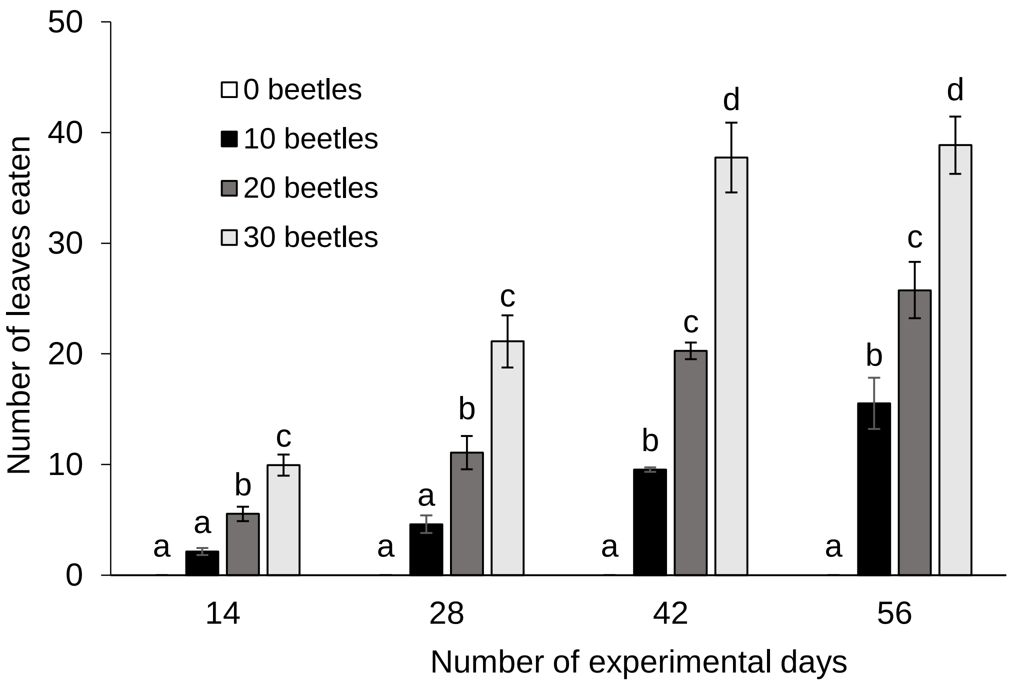

Figure 3: Average ± SE number of *Parthenium hysterophorus* leaves eaten at 14, 28, 42 and 56 days after beetle release under caged conditions, treated with the release of 0, 10, 20 and 30 individual *Zygogramma bicolorata* beetles. Different letters indicate significant differences across treatments according to Tukey's HSD at *p* < 0.05

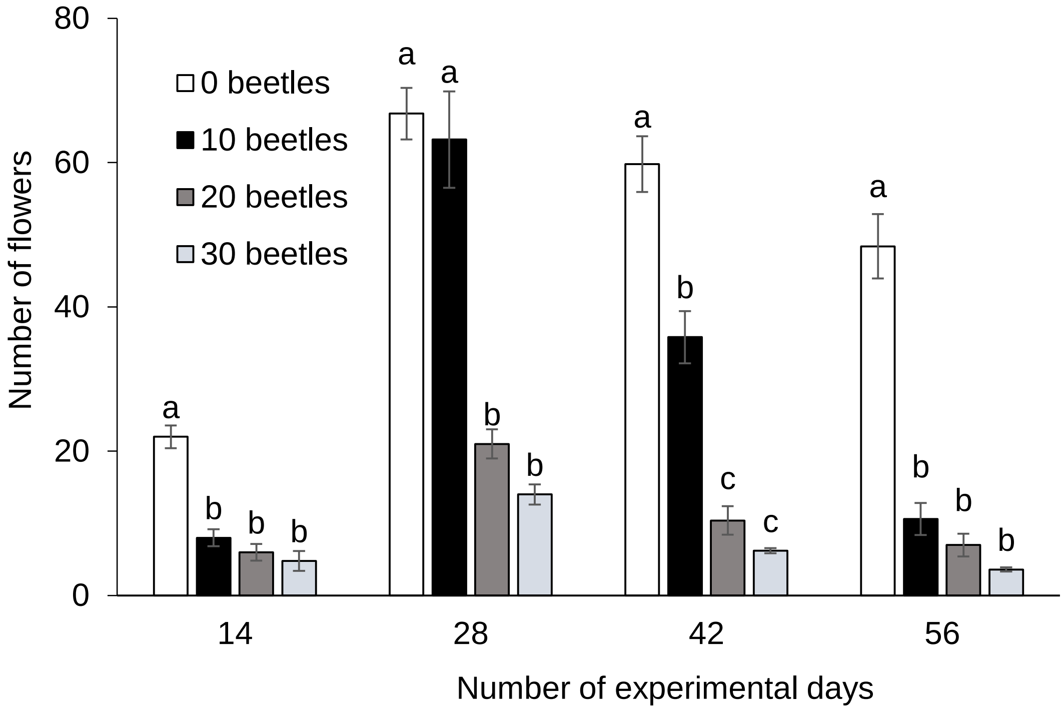

Figure 4: Average ±SE number of flowers that were still produced by *Parthenium hysterophorus* at 14, 28, 42 and 56 days after 0, 10, 20 and 30 individual *Zygogramma bicolorata* beetles were released under caged conditions. Different letters indicate significant differences across treatments according to Tukey's HSD at $p < 0.05$

Parthenium hysterophorus height and biomass were reduced with the lowest beetle number and reduced even greaterwith increasingbeetle population. Average height of *P. hysterophorus* plants ranged from an average of 30 cm in the control treatment to approximately 5 cm in the 30 beetle treatment. Heights were significantly reduced by 47% (10 beetles), 72% (20 beetles) and 87% (30) compared to the control at $F_{3,8} = 164.7, p < 0.001$ (Fig. 5a). Similarly, the fresh above-ground biomass of *P. hysterophorus* subjected to *Z. bicolorata* treatments was significantly and progressively reduced with increasing individuals of *Z. bicolorata.* The *Parthenium hysterophorus*biomass was significantly reduced by 44% (10 beetles), 73% (20 beetles) and 91% (30 beetles), compared to the the control treatment at $F_{3,8}= 100.5, p < 0.001$ (Fig. 5b). Number of seeds germinated in all plots differed significantly after seven days at $F_{3,8} = 91.7, p < 0.001$ (Fig. 6), with the highest number of germinated seed recorded in control plots after 21 days at $F_{3,8} = 2345.8, p < 0.0001$ (Fig. 6). In the subsequent period (49 and 56 days), number of seed germinating differed significantly with no seedlings observed in the plots of 20 and 30 beetles ($F_{3,8} = 1114.2, p < 0.001; F_{3,8} = 95.5, p < 0.001$). There was a strong significant differenceamong week, treatment and week by treatment (Table 4).

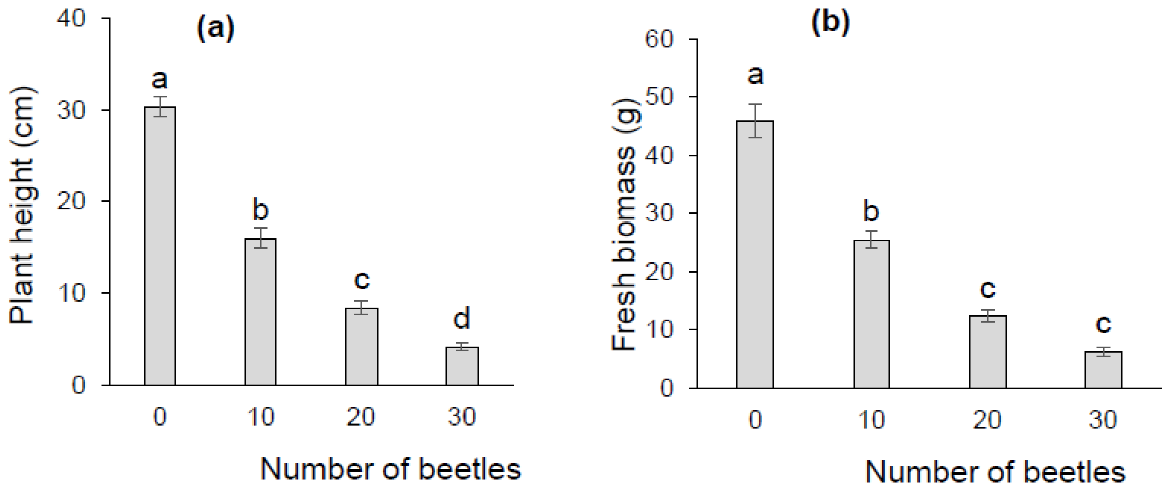

Figure 5: Average ±SE (a) height and (b) fresh biomass after being treated with *Zygogramma bicolorata* under different treatments (30, 20, 10 and 0 individual beetles) for 56 days. Different letters indicate significant differences according to Turkeys HSD at p < 0.05

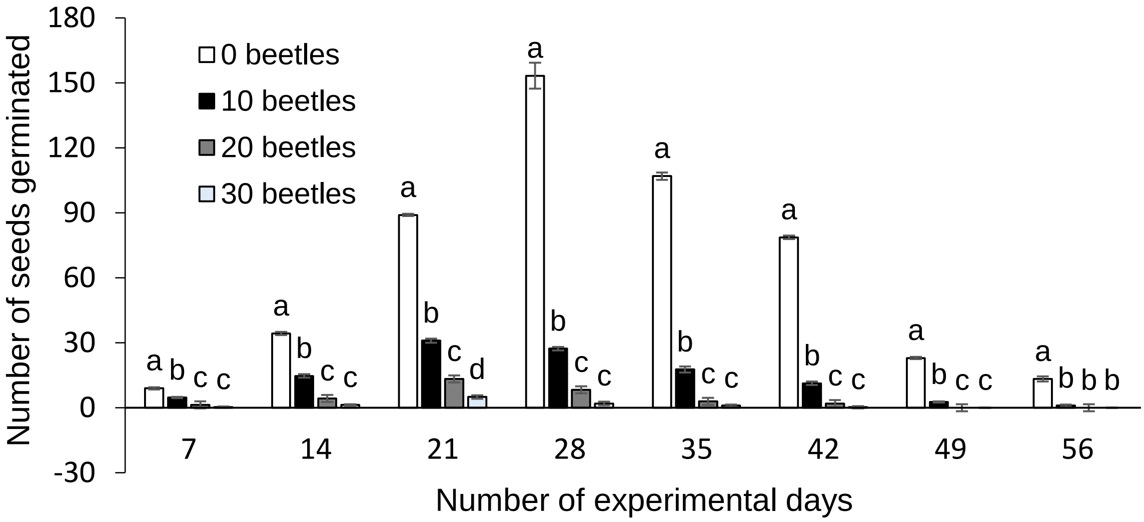

Figure 6: Average ±SE number of *Parthenium hysterophorus*seeds germinated at 7-56 days under cage conditions treated with 0, 10, 20 and 30 beetles. Different letters indicate significant differences across treatments according to Tukey's HSD at p < 0.05

4.1.2 Number of eggs, larvae and adult produced

The largest number of eggs were recorded in the highest beetletreatment14 days after beetles were released, while largest number of larvae were recorded 42 days in the highest beetle treatment and the largest number of adults were recorded after 56 days in the highest beetle treatment. There was a strong significant difference among week, treatment and week by treatment (Table 4). Fourteen days after beetle introduction, the number of eggs and released adults differed significantly across treatments at $F_{3,8} = 95.1, p < 0.001$; $F_{3,8} = 481.7, p < 0.001$ (Table 5) but not the number of larvae ($F_{3,8}= 2.32, p= 0.137$). At 42 days after beetle release, the treatment with the highest beetle treatment (30 individuals) resulted in the highest number of larvae ($F_{3,8} = 740.2, p < 0.001$) while the highest number of adults were recorded after 56 days for the same beetle treatment. There was a significant difference across treatments at $F_{3,8} = 41.9, p < 0.001$ (Table 5).

Table 4: Repeated measure analysis showing results for each mixed model of number of eggs, larvae, adult and seedbank including week, treatment and week by treatment as a fixed effects, cages as a random effect

	Eggs		**Larvae**	
	F statistics	***p***	***F statistics***	***p***
Week	$F_{2,9} = 179$	< 0.001	$F_{2,9} = 501$	< 0.001
Treatment	$F_{3,27} = 428$	< 0.001	$F_{3,27} = 248$	< 0.001
Week*treatment	$F_{6,27} = 110$	< 0.001	$F_{6,27} = 106$	< 0.001
	Adult		**Seedbank**	
Week	$F_{2,9} = 130.3$	< 0.001	$F_{7,16} = 540$	< 0.001
Treatment	$F_{3,27} = 111.8$	< 0.001	$F_{3,48} = 4396$	< 0.001
Week*treatment	$F_{6,27} = 79.4$	< 0.001	$F_{21,48} = 381$	< 0.001

Table 5: One-way ANOVA test on the number of eggs and larvae produced after *Z. bicolorata* release at 14, 28, 42 and 56 days under screen house condition. Different letters across columns indicate significant differences according to Tukey's HSD at *p* < 0.05

Eggs		**Treatment (# beetles)**					
	0	**10**	**20**	**30**	$F_{(3,8)}$	***p***	**% CV**
14 days	0^{a}	101^{b}	133^{c}	159^{d}	95.11	< 0.001	8.6
28 days	0^{a}	20^{b}	43^{c}	50^{c}	57.46	< 0.001	14.7
42 days	0^{a}	10^{b}	27^{c}	39^{d}	732.51	< 0.001	4.6
56 days	0^{a}	6^{a}	17^{b}	21^{b}	17.80	< 0.001	21.9
Larvae		**Treatment (# beetles)**					
	0	**10**	**20**	**30**	$F_{(3,8)}$	***p***	**%CV**
14 days	0^{a}	5^{a}	8^{a}	22^{a}	2.32	0.137	111.2
28 days	0^{a}	5^{a}	15^{b}	28^{c}	146.80	< 0.001	15.7
42 days	0^{a}	9^{b}	25^{c}	33^{d}	740.21	< 0.001	5.5
56 days	0^{a}	11^{ab}	16^{b}	21^{b}	9.16	0.013	44.5
Adult		**Treatment (# beetles)**					
	0	**10**	**20**	**30**	$F_{(3,8)}$	**P**	**%CV**
14 days	0^{a}	3^{b}	13^{c}	21^{d}	481.71	< 0.001	7.0
28 days	0^{a}	5^{a}	15^{b}	28^{c}	146.82	< 0.001	15.0
42 days	0^{a}	10^{b}	161^{c}	237^{d}	1140.51	< 0.001	4.6
56 days	0^{a}	115^{b}	193^{c}	270^{d}	41.92	< 0.001	16.6

4.1.3 *Cassia auriculata* leaves and bark effects on *P. hysterophorus*

All *P. hysterophorus* measures were reduced compared to the control, even at the lowest concentration treatment and measures were even lower when concentration of the plant extract increased. Root length of *Parthenium hysterophorus* treated with CaL and CaB was halved at concentrations of 30% and even further reduced under higher concentrations (Fig. 7, Table 6). Shoot length of *P. hysterophorus* treated with highest concentrations of CaL was more than three times lower compared to those treated with low concentration while CaB shown no significant difference (Fig. 7, Table 6). Fresh biomass of *Parthenium hysterophorus* treated with highest concentration of CaL was 87% lower compared to those treated with low concentration while CaB was halved at concentrations of 30% but were three times lower when concentration increased (Fig.7, Table 6). Dry biomass of *P. hysterophorus* treated with CaL was 89% lower compared to those treated with low concentration while CaB was halved at concentrations of 30% but were more than three times lower when CaL concentration increased (Fig. 8, Table 6). Total chlorophyll content of *Parthenium hysterophorus* treated with CaL was 90% lower compared to those treated with low concentration, while CaB was significantly halved at concentrations of 30% but were 71% lower when CaL concentration increased (Fig. 8, Table 6).

Table 6: One-way ANOVA test showing F statistics and P value for root length, shoot length, fresh biomass, dry biomass and total chlorophyll after being treated with CAL, CaB

Measured parameters	***Cassia auriculata leaves extracts***		***Cassia auriculata* barks extracts**	
	$F_{3,8}$	***p value***	$F_{3,8}$	***p value***
Root length	230.7	< 0.001	234.9	< 0.001
Shoot length	222.7	< 0.001	143.2	< 0.001
Fresh biomass	144.1	< 0.001	61.5	< 0.001
Dry biomass	144.79	< 0.001	51.33	< 0.001
Total chlorophyll	207.8	< 0.001	77.15	< 0.001

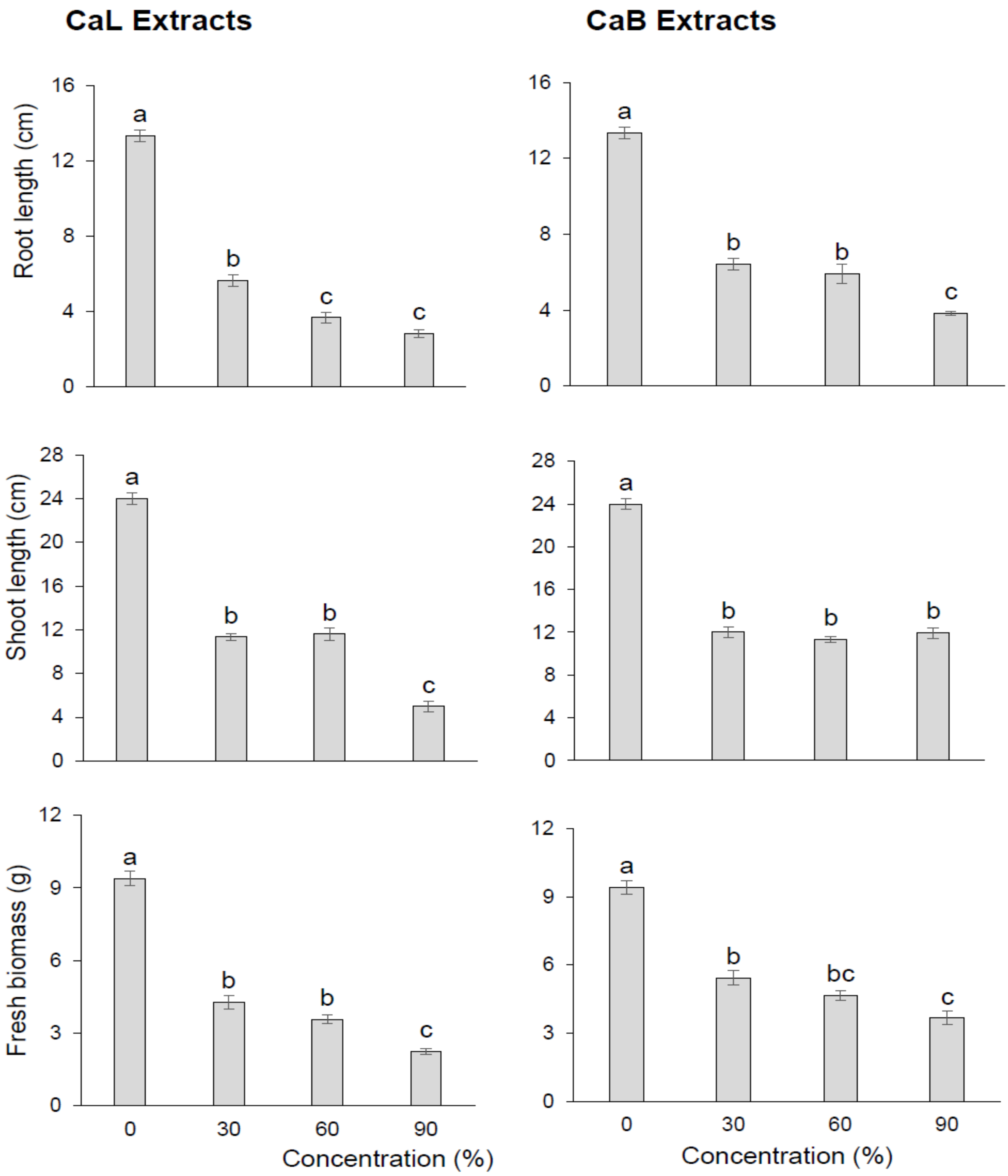

Figure 7: Average ±SE of root length, shoot length and fresh biomass after being treated with different concentrations of *Cassia auriculata* leaves (CaL) and *Cassia auriculata* barks (CaB) after 28 days in the screen house. Different letters indicate significant differences according to Turkey's HSD at $p < 0.05$

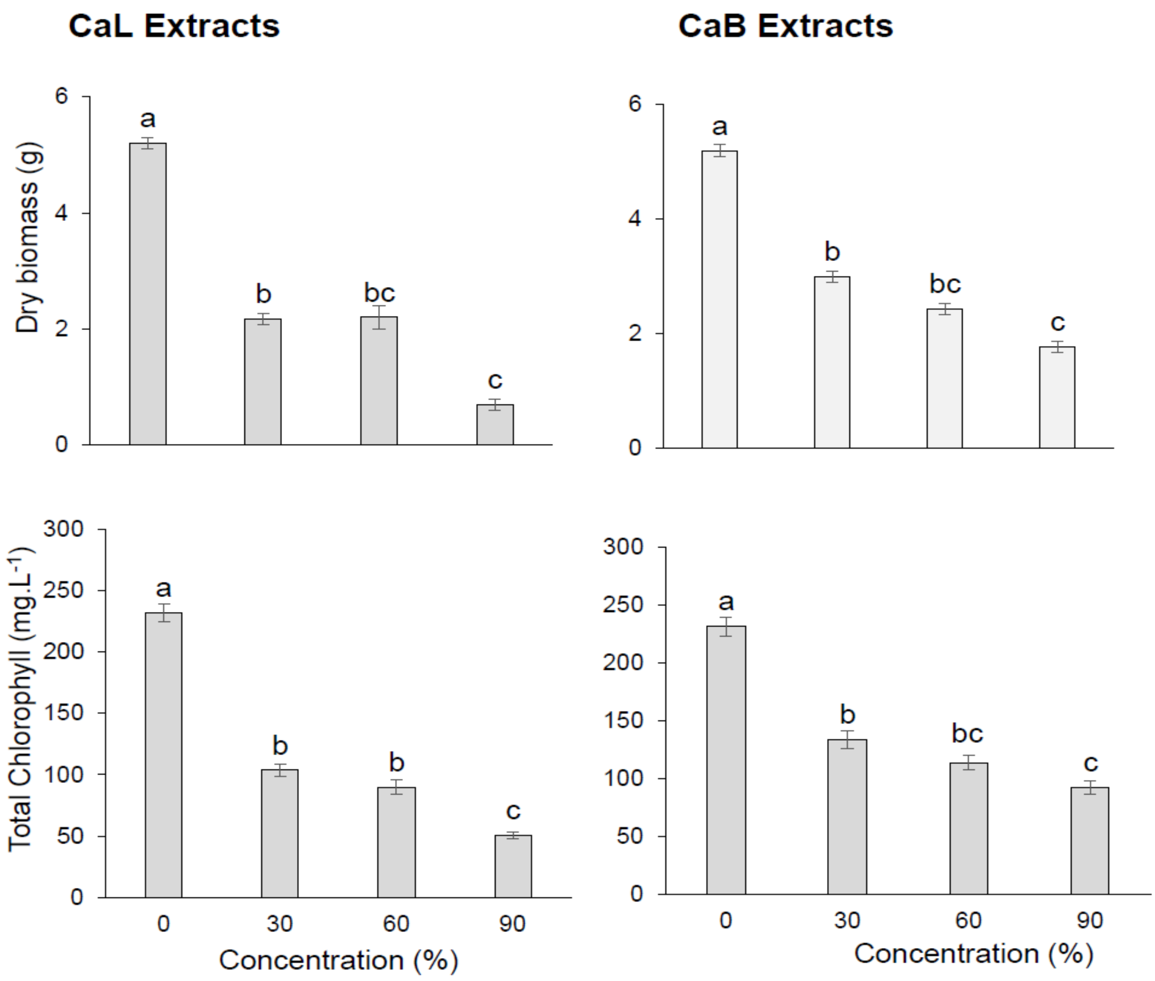

Figure 8: Average ±SE of dry biomass and total chlorophyll content after being treated with Cassia *auriculata* leaves (CaL) and *Cassia auriculata* barks (CaB) after 28 days in the screen house. Different letters indicate significant differences according to Tukey's HSD at *p* < 0.05

4.1.4 *Dovyalis caffra* leaves, fruits and 2,4-D dimethylamine effects

Application of *D. caffra* leaves and fruits extract halved *P. hysterophorus* measures at lowest concentration but when concentration of fruits increased to 1.5 g l^{-1} the effects were similar to 2,4-D 10 ml l^{-1}. Application of the lower concentration of *Dovyalis caffra* fruits (DcF) and *Dovyalis caffra* leaves (DcL) halved *P. hysterophorus* root length but high concentration of fruits reduced root length by 87% (Fig. 9, Table 7). Similarly, shoot length was halved at the lower concentration and when the concentration increased shoot length decreased by more than three times (Fig. 9, Table 7). Moreover, fresh biomass of *P. hysterophorus* was significantly reduced by 98% after application of high concentration of DcF while the lower concentration led to half the fruit production (Fig. 9, Table 7). Furthermore, *P. hysterophorus* dry biomass was halved at lower concentration and was reduced by 98% at the high concentration (Fig. 10, Table 7). Application of lower concentration of DcL and DcF halved total chlorophyll content and high concentration of DcF reduced it by 99% (Fig. 10, Table 7). The chemical herbicide 2,4-D resulted in the same control as the high concentration of DcF whereby *P. hysterophorus* root length, shoot length, fresh biomass, dry biomass and total chlorophyll content was significantly totally reduced (Fig. 10, Table 7).

Table 7: One-way ANOVA test showing *F statistics* and *P value* for root length, shoot length, fresh biomass, dry biomass and total chlorophyll after being treated with DcL, DcF and 2,4-D

Measured parameters	*Dovyalis caffra leaves extracts*		*Dovyalis caffra* fruits and 2,4-D	
	$F_{3,8}$	*p value*	$F_{3,8}$	*p value*
Root length	208.7	< 0.001	62.58	< 0.001
Shoot length	43.6	< 0.001	274.5	< 0.001
Fresh biomass	71.23	< 0.001	367.43	< 0.001
Dry biomass	61.35	< 0.001	71.08	< 0.001
Total chlorophyll	73.13	< 0.001	504.11	< 0.001

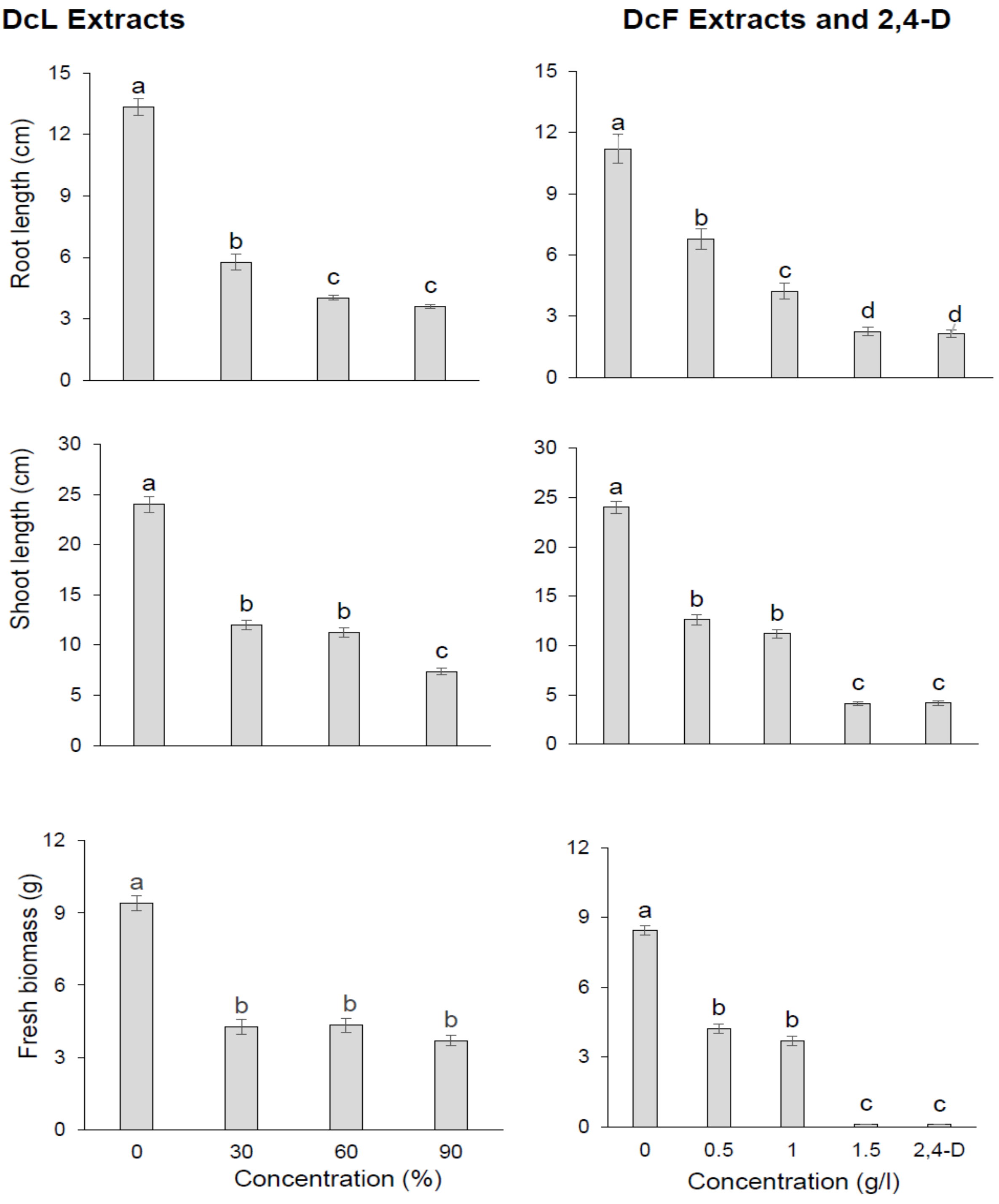

Figure 9: Average ±SE of root length, shoot length and fresh biomass after being treated with *Dovyalis caffra* leaves (DcL), *Dovyalis caffra* fruits (DcF) and 2,4-D (10 m l^{-1}) after 28 days in the screen house. Different letters indicate significant differences according to Turkey's HSD at $p < 0.05$

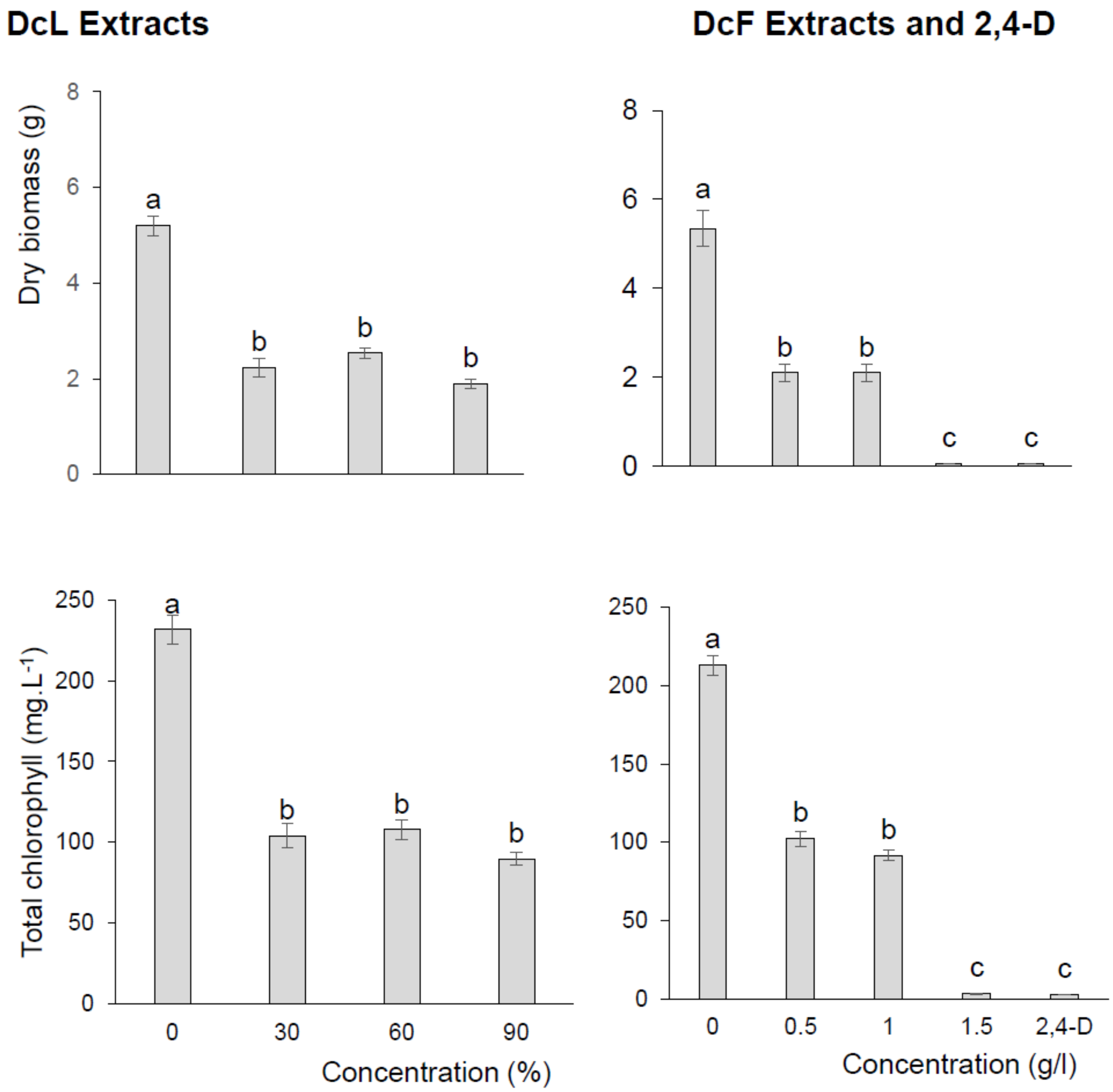

Figure 10: Average ±SE of dry biomass and total chlorophyll content after being treated with *Dovyalis caffra* leaves (DcL), *Dovyalis caffra* fruits (DcF) and 2,4-D (10 ml^{l-1}) after 28 days in the screen house. Different letters indicate significant differences according to Turkey's HSD at $p < 0.05$

4.1.5 Percentage weed control

The highest concentration of CaL (90%) resulted in the highest mean percentage of weed control at 28 days after application (85%, Table 8) compared to the low concentration treatment. Highest concentration of *D. caffra* fruits (1 g $l^{-1}$and 1.5 g l^{-1}) resulted in the highest mean percentage of weed control (88% and 87%), while 2,4-D had 100% weed control (Table 8). Percentage weed control of CaL differed significantly compared to the control at $F_{3,8}$ = 197.7, p = 0.01 (Fig. 11). Similarly, CaB and DcF resulted into a significant difference compared to control CaB ($F_{3,8}$ = 204.9, p < 0.001) and DcL at $F_{3,8}$ = 214.62, p < 0.001 (Fig. 11). In addition, DcF and 2,4-D resulted into a significant different compared to control at $F_{4,15}$ = 81.08, p < 0.001 (Fig. 11).

Table 8: Percentage weed control of *P. hysterophorus* after being treated with (CaL, CaB, DcL, DcF and 2, 4-D extracts) for 28 days in the screen house

Treatments	Dosage	Percentage Weed Control
Control	0	0
CaL	30%	79
CaL	60%	82*
CaL	90%	85*
CaB	30%	55
CaB	60%	70
CaB	90%	81
DcL	30%	51
DcL	60%	76
DcL	90%	81
DcF	0.5 g l^{-1}	83*
DcF	1 g l^{-1}	88*
DcF	1.5 g l^{-1}	97*
2, 4-D (Standard)	10 ml l^{-1}	100*

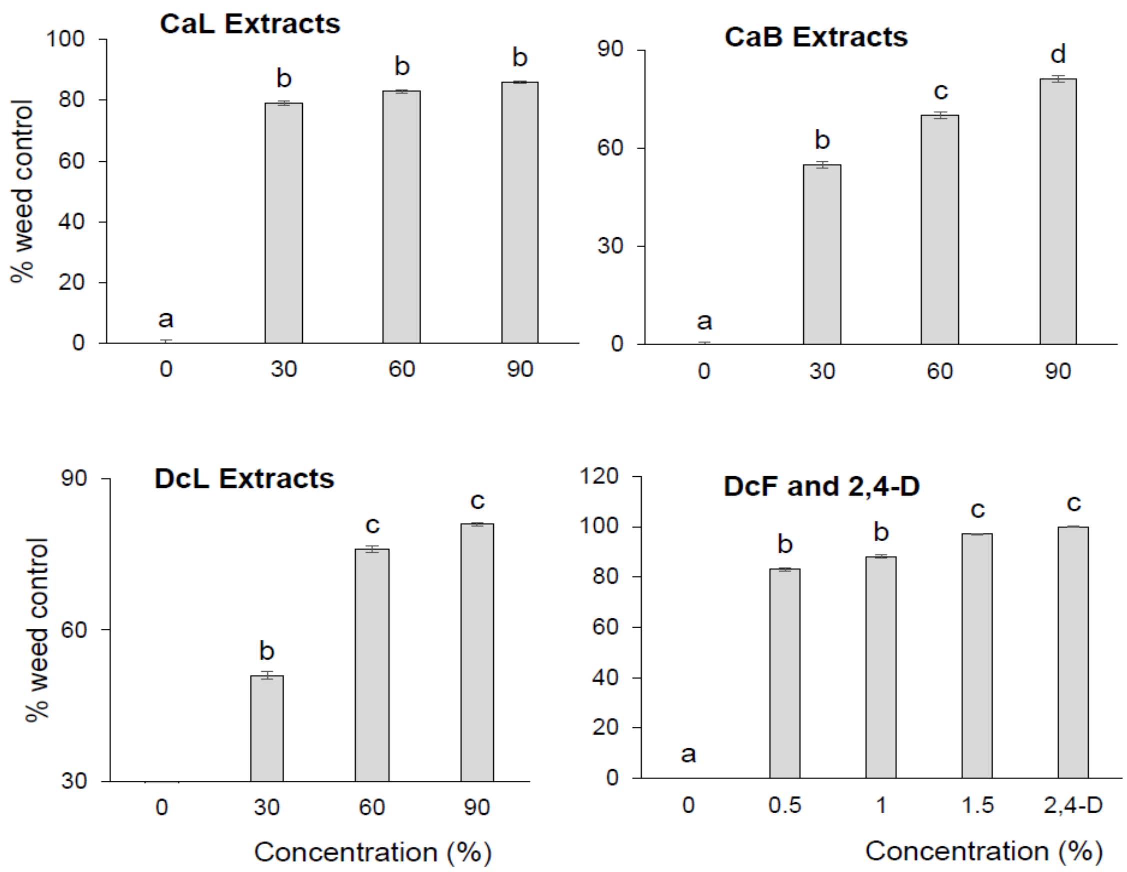

Figure 11: Average percentage weed control ± SE of CaL, CaB, DcL and DcF and 2,4-D (10 ml l^{-1}) after 28 days in the screen house. Different letters indicate significant differences according to Tukey's HSD at p < 0.05

4.1.6 Characteristics of community respondents

The majority of interviewees were male (Table 9, 10). Pastoralists (average 51-60) were older than farmers (41-50) by about 10 years (Table 9, 10). Average household size for farmers was 5-8 individuals in Mbuguni, Olasiti and Murieti, while in Sepeko the household size was larger in average compared to other areas (Table 9). Pastoralist household sizes were 4-9 individuals in Mbuguni, Olasiti, Sepeko and Murieti (Table 10). Most farmers had a primary school education with average primary school completion between 27% and 65% in Mbuguni, Olasiti and Murieti. In contrast, in Sepeko 41% of respondents had no formal education (Table 9). Pastoralist education was similar with primary school completion between 28% and of 64%, in Mbuguni, Olasiti and Murieti, while in Sepeko, 41% acquired some level of primary school (Table 10).

Table 9: Farmer interviewees socio-economic characteristics in Mbuguni, Murieti, Olasiti and Sepeko wards

Variable	Mbuguni N = 31	Olasiti N = 30	Sepeko N = 32	Murieti N = 30	Mean	χ^2	*p*
Gender						1.8	0.61
Male	55	57	59	43	54		
Female	45	43	41	57	46		
Age						92.8	0.002
21-30	-	6	-	-	6		
31-40	16	27	13	27	20		
41-50	26	30	38	30	31		
51-60	48	30	44	33	39		
60+	10	7	6	10	8		
Family Size						114.4	0.001
<=1	6	-	-	-	6		
1-4	32	23	3	-	20		
5-8	61	70	13	10	38		
9-12	-	7	31	67	35		
13-16	-	-	19	23	21		
17-20	-	-	31	-	31		
24+	-	-	3	-	3		
Level of Education						52.1	0.001
No formal education	-	10	41	7	19		
Some primary school	26	17	19	17	19		
Completed primary school	65	27	38	63	48		
Some secondary school	3	23	-	7	11		
Completed secondary school	6	20	3	7	9		
Post-secondary qualification	-	3	-	-	3		

Table 10: Pastoralist socio-economic characteristics in Mbuguni, Murieti, Olasiti and Sepeko wards

Variable	Mbuguni N = 33	Olasiti N = 32	Sepeko N = 32	Murieti N = 31	Mean	χ^2	*p*
Gender						2.8	0.421.
Male	64	50	59	45	55		
Female	36	50	41	55	45		
Age						110.2	0.001
<=30	-	-	-	3	3		
31-40	6	12	19	23	15		
41-50	24	56	53	10	36		
51-60	60	31	25	48	41		
61-70	6	-	3	16	8		
71+	3	-	-	-	3		
Family Size						75.8	0.003
<=3	9	3	-	3	5		
4-9	84	88	47	97	79		
10-15	6	9	44	-	20		
16-21	-	-	6	-	6		
22+	-	-	3	-	3		
Level of Education						54.1	0.001
No formal education	12	9	34	22	20		
Some primary school	12	16	40	6	19		
Completed primary school	64	28	25	58	44		
Some secondary school	9	25	-	3	12		
Completed secondary school	3	22	-	6	10		

4.1.7 Farmer and pastoralist knowledge of *P. hysterophorus*

All interviewees had seen and knew about *P. hysterophorus. Parthenium hysterophorus* knowledge (impacts of the weed to both agriculture and pastoralism) was significantly higher among farmers than among pastoralists ($Z = 2.220$, $p = 0.041$), but there was no difference in knowledge between women and men ($Z = 0.812$, $p = 0.581$). About 70% of all households claimed that they had seen that *P. hysterophorus* arrived in the area between 2015 and 2019, while 30% reported that it appeared earlier (by 2010). Moreover, both farmers and pastoralists claimed that the weed was most frequently found in farms, followed by road-sides, pastures, and along streams and water courses at $\chi^2 = 20.071$, $p = 0.017$ (Fig. 12). There was a strong positive **c**orrelation ($r^2 = 0.85$, $p < 0.001$) between higher level of education and people who knew about *P. hysterophorus* and its management.

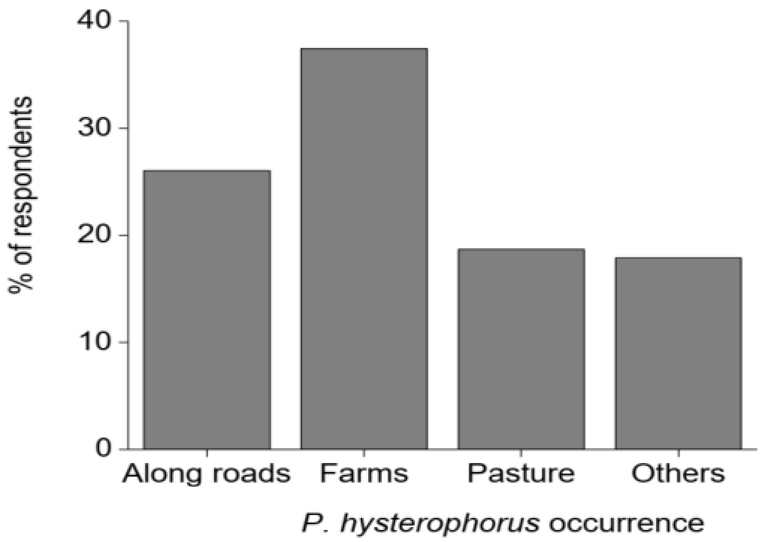

Figure 12: The proportion of farmers and pastoralists combined who claimed that *P. hysterophorus* occurred in different sites

4.1.8 Impacts of *P. hysterophorus* on agriculture

Of the surveyed farmers (N = 118), 48% depended both on farming and cattle grazing, 29% on farming alone, 21% on farming and other activities like business, motorcycle driving, carpentry and selling foods while 2% on other activities like business. Almost half mentioned that maize and bean yields have been affected and 29% mentioned the crop colour had changed (Table 11). Farmers also stated that controlling this noxious weed requires a lot of manpower. Almost half also reported that income is highly affected (Table 12).

Table 11: Number and proportion of farmers claiming that yieldswere reduced by the presence of *P. hysterophorus*

Yield reduced	Frequency	%
Maize & Beans	52	44
None	28	24
Maize	19	17
Maize & Cassava	10	9
Maize & Potatoes	9	7

Impacts on crops	Frequency	%
Don’t know	9	7
No effect	29	24
Reduced yield	17	13
Reduced soil fertility	29	25
Makes maize yellow	34	29

Table 12: Number and proportion of farmers that claim impacts of *P. hysterophorus* on yield and how their income has been affected by the weed

Yield reduction	Frequency	%
<25% reduction	6	4
25% reduction	32	26
50% reduction	26	21
75% reduction	22	18
>75% reduction	7	6
None	30	24

Impact on income	Frequency	%
Fluctuates over time	10	8
Not affected	21	17
Highly affected	57	46
Don’t know	6	4
Slightly affected	29	23


Plate 6: Farmers explaining the impacts of *P. hysterophorus*

4.1.9 Impacts of *P*. *hysterophorus* on livestock

Pastoralists reported that their livestock accidentally graze on *P*. *hysterophorus* during both dry and wet seasons (55%), in the dry season only (35%), or the wet season only (2%), while 9% claimed that livestock never eat this plant. There was a significant difference on perceived *P*. *hysterophorus* impacts among wards ($\chi^2 = 39.55$, $p < 0.001$), and the majority of pastoralists in Sepeko did not know the impacts *P*. *hysterophorus* have on their livestock (Fig.13). Of all pastoralists,74% claimed that *P*. *hysterophorus* reduces milk quality, while 22% did not observe any difference and 5% did not know. In addition, 30% mentioned that the milk turns either green and yellow after cattle had ingested *P*. *hysterophorus*. Furthermore, 60% of pastoralists claimed that milk quantity declined. However, no change in livestock fertility was observed by the vast majority of pastoralists. Other impacts on the physical condition of livestock included mouth diseases (11%) or hair loss (14%).

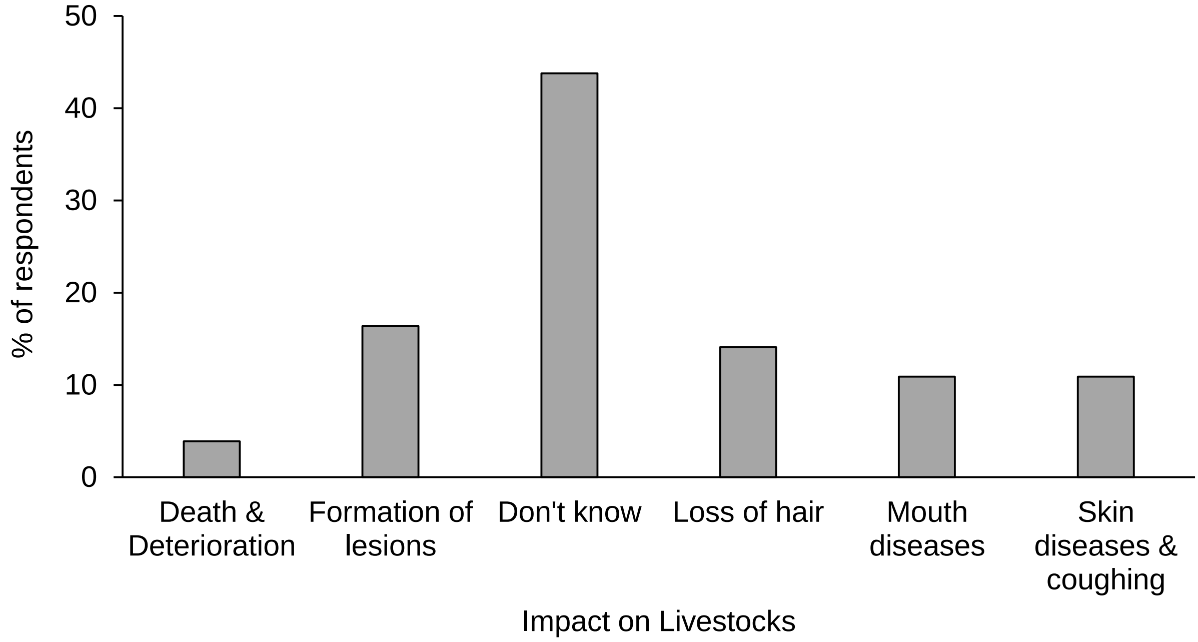

Figure 13: The proportion of pastoralists mentioning how *P. hysterophorus* affects their livestock health

Plate 7: Pastoralists explaining the impacts of *P. hysterophorus*

4.1.10 Impacts of *P. hysterophorus* to humans and current control mechanisms used

Of all 251 respondents, four major impacts of *P. hysterophorus* on human health were mentioned: Allergic reactions (24%), influenza and coughing (23%), itching skin and wounds (20%) and nose bleeding (19%), while only 8% claimed that there was no impact on health. *Parthenium hysterophorus* impacts were perceived differently by farmers versus pastoralists ($\chi2$ = 50.20, p = 0.02): Farmers claimed that the weed causes skin itching while the pastoralists emphasized influenza and coughing (Plate 8). Both farmers and pastoralists reported that the most often applied methods in controlling this weed were mainly uprooting and slashing (Fig. 14). However, these methods differed significantly between farmers and pastoralists (χ^2 = 71.32, p = 0.003): Farmers controlled *P. hysterophorus* both by uprooting and slashing while pastoralists mostly only slashed (Fig. 14). Famers also burned more frequently however, all respondents reported that the currently applied measures have not been successful, can result in health problems and demand high manpower. Of both farmers and pastoralists (N = 251), only 22% were aware of the use of the biocontrol agent (*Z. bicolorata*).

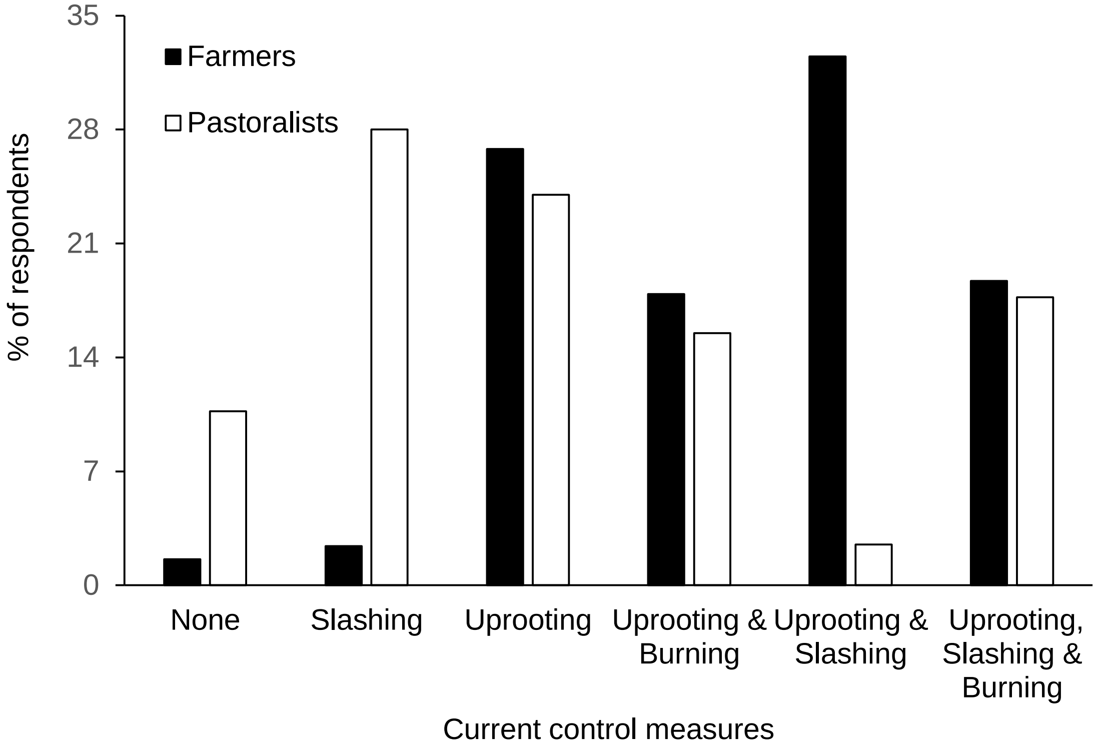

Figure 14: Current control measures which are used to control *P. hysterophorus* by both pastoralists and farmers

Plate 8: "A" impact of *P. hysterophorus* to human and "B" uprooted *P. hysterophorus* ready for burning

4.1.11 Biological control using *Z. bicolorata*

This study surveyed the areas where *Z. bicolorata* was introduced and where it was not introduced as a control. The results revealed that in areas where the beetle *Z. bicolorata* had been released, *P. hysterophorus* plant population size was significantly reduced (39.2 ± 2.3 individuals) compared to the areas without beetles (45.6 ± 2.1 individuals; t = 1.9; df = 78; p = 0.05). Similarly, the average height of *P. hysterophorus* plants was significantly reduced to almost one third in areas with *Z. bicolorata* (10.2 ± 0.3 cm) compared to the areas without the beetle (32.1 ± 0.6 cm; $t = 31.5$; $df = 61$; $p < 0.001$). Furthermore, the number of broad-leaved forbs was about four times higher in the area with *Z. bicolorata* (14.6 ± 2 individuals) compared to the areas without *Z. bicolorata* (3.2 ± 1.1 individuals; $t = 7.8$; $df = 43$; $p < 0.001$). The same pattern was visible for the number of grass plants, which was significantly higher in the area with *Z. bicolorata* (9.2 ± 1.1 individuals) compared to the area without *Z. bicolorata* (2.3 ± 1.2 individuals; $t = 7.01$; $df = 42$; $p < 0.001$). The number of sedges also differed significantly with a higher number in the area controlled with *Z. bicolorata* (10.7 ± 2.2 individuals) compared to the area without the biocontrol agent (1.8 ± 1.1 individuals; $t = 5.8$; $df = 40$; $p < 0.001$).

4.2 Discussion

As with many other invasive species, an effective integrated approach to control *P. hysterophorus* is highly needed. Both methods (use of biocontrol insect *Z. bicolorata* and plant extracts) are individually effective in different ways, so it is believing that combining them with an integrated approach is promising. Defoliation by *Z. bicolorata* from the early stages of plant growth was largely responsible for the reduction in plant vigour. Additionally, the herbicidal extracts of *C. auriculata* and *D. caffra* are highly effective against germination and growth of *P. hysterophorus.* Extracts of all tested natural herbicides exhibited phytotoxic activity against early seedling growth of the noxious weed *P. hysterophorus.* Lastly, *P. hysterophorus* has infested a range of landscapes and affected the crop and livestock production, animal health, human health and livelihood of predominantly subsistence farmers across the surveyed wards. Mechanical measures have been used to control *P. hysterophorus.* However, because *P. hysterophorus* is such a difficult invasive to control and since touching it can have impacts to human health, it will be practical a focus on biocontrol integrating beetles and plant extracts. Specifically, this study recommends the use of beetles at densities of at least five male and female individuals per meter square combined with extracts of *D. caffra* and *C. auriculata*.

4.2.1 Effectiveness of *Z. bicolorata* on *P. hysterophorus* vegetative growth

Defoliation caused by the biocontrol agent *Z. bicolorata* had an immediate adverse effect upon *P. hysterophorus* performance. Defoliation imparts a severe physiological stress on plants by suddenly restricting the availability of photosynthetic needed for growth. Young plants, and plants that have not yet developed a storage reserve system, suffer most from this sudden stress

(Prins & Verkaar, 1992). However, the beetle was only effective early in the experiment and later, little herbivory was seen. Similar results were reported by Shabbir *et al.* (2016), were the study found complete defoliation after applying three pairs of *Z. bicolorata* to one young *P. hysterophorus* individuals.

Heavy defoliation of *P. hysterophorus* caused by adults and larvae of *Z. bicolorata* resulted in weakening the plants and reducing flower production and seedbank. Although *Z. bicolorata* does not feed on *P. hysterophorus* flowers, a reduction in the number of flowers and seedbank is associated with the feeding behavior of larvae, which tend to congregate and feed on tender leaves, terminal and axillary buds and, thereby, lead to stunted growth and fewer flowers and low seeds productivity (Manjunath, 2010; Mcconnachie, 2015). Manjunath (2010) reported that 98% of flowers were reduced when *P. hysterophorus* plants were heavily skeletonized by *Z. bicolorata* in India. In Queensland, Australia, a total of 400 000 adult *Z. bicolorata* were released to control *P. hysterophorus* (Dhileepan & Strathie, 2009); this release resulted in 86% reduction of flowers and 78% reduction in seedbank which is similar to our results. Lower flower production translates into a decreased soil seed bank and consequently lower *P. hysterophorus* density.

Defoliation by *Z. bicolorata* also significantly reduced plant height and biomass. This might be associated with continuous feeding by *Z. bicolorata* on the vegetative apical meristems, resulting in reduced primary stem height and altered branching pattern (Dhileepan *et al.*, 2000). *Parthenium hysterophorus* was heavily skeletonized by *Z. bicolorata*. Plants with few leaves are poor competitors for light and are more susceptible to petal and sepal abscission, which results in poor nectar availability and, hence, inhibit pollination (Saifuddin *et al.,* 2010) as well as reduced biomass (Falster & Westoby, 2003). Jayanth (1987) found that, plant height and biomass were reduced 65% and 89%. Similarly, Shabbir *et al.* (2016) found that shoot height and dry biomass were significantly compromised after introduction of high *Z. bicolorata* densities.

4.2.2 Success of *Z. bicolorata* as a biocontrol programme

Smith and Debach (1942) describe criteria to measure the success of a biocontrol programme: a) reduction in the pest populations b) maintenance of pest populations at a low level following the establishment of natural enemies and c) a high rate of survival of the pest populations when protected from their natural enemies. Results from controlled experiment, shows that *Z.*

bicolorata meets the first and third criteria as a biocontrol agent against *P. hysterophorus* since the beetles reduced plant height, damaged leaves, and reduced the number of flowers, i.e., the potential future generation. This study also confirmed that *Z. bicolorata* was capable of defoliating *P. hysterophorus* within a relatively short period of time, particularly when the beetles were released at high population densities. Singh *et al.* (2017) confirmed that both larvae and adult *Z. Bicolorata* feed on the leaves of *P. hysterophorus* and that the beetles complete their life cycles exclusively on *P. hysterophorus* plants. The use of the biocontrol agent *Z. bicolorata* is recommended as an appropriate method of controlling *P. hysterophorus* in Nepal and India (Jayanth & Bali, 1993; Manjunath, 2010; Chaudhari *et al.*, 2012), Southern Asia (Dhileepan & Senaratne, 2009), Australia (Dhileepan, 2001) and South Africa (Strathie, 2015b). Therefore, this study recommends that, beetles should be introduced at densities of at least five male and female individuals per square meter for high effectiveness.

In addition, (Adkins & Shabbir, 2014) also recommended *Z. bicolorata* as an environmentally friendly biocontrol method, because they does not impact non-targeted species and seems to be a sustainable in *P. hysterophorus* control (Chaudhari *et al.*, 2012). *Zygogramma bicolorata* are capable of digging into the soil at about 3 cm depth for more than six months in the absence of *P. hysterophorus* and emerge with the onset of monsoon (Jayanth & Bali, 1995). Jayanth and Bali (1995) reported *Z. bicolorata* are active during the wet season while long dry spells can significantly reduce the beetle population under field conditions. Although many consider the use of biocontrol agents as safe, other studies have reported biocontrol agents switching to native plant species (Louda *et al.*, 1997). These authors reported that the weevil *Rhinocyllus conicus,* which were introduced to control exotic thistles, exhibited an increase in host range and reduced the abundance of neighboring native plants. Therefore, it will be practical to conduct a proper field study to assess whether *Z. bicolorata* feeds on other plants species when *P. hysterophorus* is depleted in Tanzania.

4.2.3 Effectiveness of *Z. bicolorata* in the field where it was introduced

Not only did *Z. bicolorata* control the invasive *P. hysterophorus,* but it also increased plant diversity of native species at the release site: There was a significant increase in the number of broad-leaved forbs, grasses and sedges compared to the control. The high density of *P. hysterophorus* plants and lack of other plant species in the area without *Z. bicolorata* ,may be associated with allelochemical interference or competition (Dogra *et al.*, 2011). Conversely, the displacement ability of *P. hysterophorus* may be because they are better adapted to changing environmental conditions than native species (Khan *et al.*, 2010). Parker and Reichard (1998) found that the presence of alien species can threaten the persistence of native species possibly because of the negative effects of competition from the invasive alien on native species populations. Allelochemicals work interdependently and their effects have been observed in both crop reduction and animal production due to *P. hysterophorus* (Dogra *et al.*, 2011). Once these allelochemicals are dispersed in the soil, they may come into contact with other physical, chemical, biological and entities which may influence activity of allelochemicals and therefore either amplify or reduce their impact on recipient plants (Blum, 1996). Shabbir and Bajwa (2006) report a 90% reduction of herbaceous components of vegetation due to the destructive nature of the *P. hysterophorus* and its allelopathic effects. Additionally, Msafiri *et al.* (2013) found that leaf and seed aqueous extracts of *P. hysterophorus* significantly reduced *Chloris gayana* (a grass) and *Alysicurpus glumaceae* (legumes species) under laboratory condition. Therefore, it will be practical to conduct the study in areas with high abundance of *P. hysterophorus,* so as to identify the reasons behind its invasiveness.

4.2.4 Effectiveness of CaL, CaB, DcL, DcF and 2,4-D on *P. hysterophorus* growth

Percentage seedling growth of *P. hysterophorus* were delayed and inhibited or reduced significantly by plant extracts of *C. auriculata*, *D. caffra* and also by the herbicide 2,4-D. All *P. hysterophorus* growth parameters were suppressed at even low concentration, but even further at high treatment concentrations. While the chemical herbicide 2,4-D was as effective as the best result from the plant extracts (DcF), use of natural herbicides may avoid the negative impacts to human health and the environment seen with chemical herbicides (Soltys *et al.*, 2013).

Seedling height are important in determining seedling vigor and chlorophyll content is the crucial plant component that determines the plant's ability to perform photosynthesis (Westoby *et al.*, 2002). While a low chlorophyll content has been associated with a plant's failure to compete for light and thus, to survive (Falster & Westoby, 2003), seedling height and fresh biomass are as well important in ensuring seedling's competitiveness. Short seedlings cannot compete well for light while those with low fresh biomass are more susceptible to the effects of trampling and other physical factors within their environment. Therefore, plant extracts' ability to affect these three parameters negatively can help in controlling the spread of *P. hysterophorus*.

While *P. hysterophorus* can be efficiently suppressed by chemical herbicides such as 2,4-D this study suggests using natural extracts leads to the same effect but with less harm to the environment. As the mode of action of most allelochemicals is similar to synthetic herbicides, the study suggests using *C. auriculata* and *D. caffra* extracts in *P. hysterophorus* management. Similarly, Anjum *et al.* (2005) found that aqueous extracts of grasses *Imperata cylindrica* and *Desmostachya bipinnata* not only suppress the germination and growth of *P. hysterophorus* under experimental conditions, but also reduce the spread of this noxious weed in the field.

Chemical herbicides are indispensable in controlling *P. hysterophorus* (Khaliq *et al.*, 2011), but the effective 2,4-D dosage is so high that it compromises the environment and might even trigger resistance of the target species (Datta *et al.*, 2018). Using chemical herbicides likely result in accumulation of active compounds in the soil, increase in weed species and acceleration evolution of resistant biotypes (Soltys *et al.*, 2013). For example, *Lolium rigidum, Avena fatua, Amaranthus retroflexus* became resistant after long-term application of 2,4-D (Soltys *et al.*, 2013). Since abandoning chemical weed control is, with current agricultural practices, impossible, it is necessary to create new classes of herbicides with new mechanisms of action and target sites not previously exploited that do not have the negative effects currently seen with petroleum-based chemical herbicides. Eco-friendly trends in weed management force scientists to reach for innovative sources and tools. Natural compounds pose potentials for the discovery of new environmentally safe herbicides, so called "bio-herbicides", which are based on compounds produced by living organisms. Therefore, this study suggests the use of bio-herbicides.

This study also recommends using *Cassia auriculata leaves* and *Dovyalis caffra* fruits as part of an integrated approach to *P. hysterophorus* management. *Cassia auriculata* leaves and *Dovyalis caffra* fruit extracts were comparatively more inhibitory to seedling growth of *P. hysterophorus* than corresponding bark extracts, indicating that leaves and fruits contain greater concentrations of inhibitors than does the bark (Javaid *et al.*, 2010). Noor and Khan (1994) also found greater inhibitory effect of extracts of aerial parts than sub-aerial parts. Similarly, Ngondya *et al.* (2016) found high concentrations of *Desmodium uncinatum* leaf extract reduced *Gutenbergia cordifolia's* ability to perform photosynthesis and led to stunted growth**.** Better performance of *D. caffra* fruits compared to its leaves might be associated with tannins, alkaloids, steroids and carbohydrates in its extract: Tannins, for instance, likely have a strong astringent property and antimicrobial activity (Aldaihan & Bhat, 2012). *Dovyalis* *caffra* fruits are already recognized for their herbicidal properties and is commonly used in agriculture as a weed killer (Omotayo *et al.*, 2018). Additionally, both *C. auriculata and D. caffra* have been naturalized in Tanzania can be easily grown, and have a quick generation time (Schmelzer, 2008; Chingwaru *et al.*, 2015). For these reasons, use of these species can provide an optimal tool for long-term sustainable management of *P. hysterophorus* using bio-herbicides.

Number of weed count (% weed control) in general were reduced after application of CaL herbicidal extracts and the similar results were seen in DcF and 2,4-D. Reduction in the number of weed count might be associated with the inhibitory effects *C. auriculata* and *D. caffra* have which resulted in reduction of cell division and elongation hence lower growth of *P. hysterophorus*. Although 2,4-D shown the high efficiency in reducing the weed count it will not be practical to accept it due to the effects it has in the environment (Soltys *et al.*, 2013) rather herbicidal extracts of *C. auriculata* and *D. caffra* can be used instead. It is practical to accept it because percentage weed control of CaL and DcF worked effectively according to European Weed Research Society Classification Scale. Bangi *et al.* (2014) conducted an experiment which combines herbicidal extracts of mango and glyphosate to kill the weeds in tomato farms. The study revealed that, herbicidal extracts are beneficial to keep the crop weed in low population although it cannot be compare with glyphosate which reduces the cost of weeding and keep the weed population below the economic threshold level throughout the crop growth period.

4.2.5 Farmer and pastoralist knowledge of *P. hysterophorus*

Invasions of *P. hysterophorus* are perceived to be widespread and increasing in agricultural areas, along the roads and in pasture lands. A majority of farmers indicated that *P. hysterophorus* had been in the area for long period of time. *Parthenium hysterophorus* density is high in places where the soils are disturbed frequently for the purposes of construction of roads, buildings, and waterways (Khan *et al.*, 2013a). Therefore, the high density in farms and along roadsides in the Arusha area of Tanzania might be due to farm preparation and road construction Although the farming communities were largely aware of the *P. hysterophorus* invasion, its health effects and the need for management in non-cropped areas were underestimated by some farmers. These results justify the need for better awareness around the health and financial effects of *P. hysterophorus* and immediate management plan across the wards.

Furthermore, presence of *P. hysterophorus* in pasture lands leads to large scale economic losses in the form of reduced level of animal productivity, increased herd mobility rates, more difficult stock handling and management and a considerably reduced property capital value for pastoralists. *Parthenium hysterophorus* in pastures endangers livestock and lowers their productivity (Ayele *et al.*, 2013). It reduces the quality and quantity forage and is unpalatable or even poisonous to livestock (Seta *et al.*, 2013). This study found household members who attended at least primary school were able to explain the impacts and control measures of this invasive species, similar to Kilkenny *et al.* (2016) study which recommended that education is important for ecological understanding.

4.2.6 Impact of *P. hysterophorus* agricultural yield and on livestock

Since agriculture is the backbone of Tanzania's economy (Bergius *et al.*, 2018), *P. hysterophorus* should be controlled to serve not only marginalized communities but also the entire country. *Parthenium hysterophorus* negatively impacts a wide range of crops. The impact *P. hysterophorus* has on yield and income is likely to result in poverty to local communities. Farmers in the surveyed population depend on farming and cattle grazing as the main source of income whereby the income is affected by 46%. *Parthenium hysterophorus* has effects on maize and beans, crop types that 69% of Tanzanians depend on (Tibesigwa *et al.*, 2019).

Parthenium hysterophorus is an aggressive weed and therefore poses a serious threat to livestock. It leads to declines in grass production, which leads to declines in livestock productivity (Seta *et al.*, 2013).The invasive occurs on rangelands throughout the year and has been reported to reduce pasture production and also potentially makes the land infertile (Dhileepan, 2007). In this study, 44% of pastoralists did not know exactly what impacts *P. hysterophorus* cause to their livestock, which might have been associated with level of education. They reported when livestock feed on *P. hysterophorus,* milk turns green and yellow. Patel (2011) reported the impacts of *P. hysterophorus* on livestock are edema, absence of hair and coloration on the neck and shoulders. Therefore, it is practical to conduct public awareness (capacity building and outreach programme) in the areas which is highly infested so that the impact of invasive can be reduced (Batish *et al.*, 2004). Outreach programmes and capacity building play an important role in successful management of invasive plant species (Bajwa *et al.*, 2019). A high level of awareness about *P. hysterophorus* presence, its aggressive expansion, and the ability of farmers and pastoralists to identify the weed at different growth stages is critical for *P. hysterophorus* management. Kapoor (2012) reported that most farmers were familiar with *P. hysterophorus* growth habit and morphological features, knowledge about its impacts and potential management options. Therefore, it is practical to increase the knowledge about *P. hysterophorus* in Tanzania especially in the areas where local communities are not attending schools. This should be done several times for a wide understanding.

4.2.7 Impacts of *P. hysterophorus* in humans and current control measures

A significant proportion of those interviewed reported negative effects of *P. hysterophorus* on their health while others were unaware of such effects. These results suggest a lack of education around health effects of this invasive. Farmers claimed mainly that their skin itched, which can be associated with farmland preparation, sowing, weeding and harvesting. Ayele *et al.* (2013) reported allergic dermatitis and itchy skin as well as allergic rhinitis and asthma due to inhalation of pollen, which is confirmed in this study. In previous studies conducted by Khan *et al.* (2013a) in Pakistan, a small number of farmers reported health issues caused by *P. hysterophorus*. Even more severe health effects have been reported from Australia (Chippendale & Panetta, 1994) and Ethiopia (Hundessa, 2016). These differences might be attributed to relative infestation levels, management practices and awareness level. Although little is known about the possible mechanism of these health effects, parthenin (a major secondary metabolite of *P. hysterophorus*) has been identified as a the potential chemical causing allergies and toxicity (Narasimhan *et al.*, 1977).

Most of the interviewed households in the study area are attempting mechanical control of the invasive *P. hysterophorus*. Large numbers are not aware about biological control methods even though these methods may be more effective. Several interviewees mentioned that the weed always regrows when mechanical methods are used, which was also found by Navie *et al.* (1996). Third world countries use mechanical methods because labor is cheap and often people are not aware that invasive species require more stringent methods for control (Navie *et al.*, 1996). Mechanical methods like burning and slashing can be effective when there is full engagement of the local communities through outreach programmes and capacity building (Adkins *et al.*, 2018). For example, in Nepal, public awareness of *P. hysterophorus* management has successfully been undertaken so that communities owned and participated in *P. hysterophorus* control projects (Shrestha *et al.*, 2019).

A focus on capacity building and outreach programmes in Tanzania could reduce negative effects the weed has in agricultural lands on livestocks and on humans. Economic assistance or financial incentives from the authorities along with good coordination in management efforts are critical. Knowledge about biological control and integrated management should be taught to extension officers, farmers and pastoralist for effective management.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Parthenium hysterophorus is spreading at an alarming rate in Tanzania (Kilewa & Rashid, 2014). It negatively affects crop and livestock production, animal and human health, and economic status of both farmers and pastoralist communities. An integrated approach, using several methods, is more likely to be effective than only employing one technique, especially if that technique is only mechanical control (Shabbir *et al.*, 2018). Even the use of only a single bio-control insect like *Z. bicolorata* is unlikely to control this aggressive invasive species (Shabbir *et al.*, 2018). *Z. bicolorata* and bio-herbicides can offer remedies to the impacts *P. hysterophorus* causes to people, agriculture and biodiversity. Therefore, using both of these methods should have a synergistic effect on controlling the invasive. This study found that all plant extracts tested suppressed the growth of *P. hysterophorus,* especially at higher concentrations. Furthermore, higher concentrations of CaL and DcF showed efficacy beyond the limit of acceptability according to the European Weed Research Society Classification Scale.

It will be practical to practice capacity building and outreach programme in Tanzania so as to reduce this negative effects the weed has not only in agricultural lands but also in livestocks and humans. More importantly it should be economic assistance or financial incentives from the authorities along with good coordination in management efforts so as to manage *P. hysterophorus.* Moreover, there is a need to train locals on the use *Z. bicolorata* and use these bio herbicides, while more research should be done in exploring naturalized plant extracts as part of an integrated approach in controlling *P. hysterophorus.*

5.2 Recommendations

Future studies should be conducted to more fully delineate best management practices to combat this invasive species. The following studies is recommended:

(i) Determine the combined effect of *Z. bicolorata* and other methods that are practiced in Tanzania using competitive plants and plant extracts. As with many other invasive species, *P. hysterophorus* cannot be managed by using a single method alone (Shabbir *et al.*, 2018). For example natural extracts from *C. auriculata* and *D. caffra* can be combined with the biological agent *Z. bicolorata*, or can be complemented with competitive, suppressive plants (Shabbir *et al.*, 2013).

(ii) Proper field studies that verify whether the beetles really do not feed on other plants in the wild once *P. hysterophorus* is depleted should be conducted. This will confirm the safe use of the beetle when released in the wild.

(iii) Studies should be conducted to verify if *P. hysterophorus* suppresses neighboring native plants due to competition or allelopathy.

(iv) Studies should be done to test the effectiveness of *Z. bicolorata* at different growth stages of *P. hysterophorus*, i.e., when the plant is already established in old stands or when it is first establishing in an area and plants are young.

(v) Research should be conducted to characterize CaL, CaB, DcL and DcF at the molecular level to identify bioactive compounds responsible in suppressing *P. hysterophorus*.

(vi) More studies should be done to explore additional plant extracts that suppress *P. hysterophorus*.

(vii) Basic knowledge on the ecology of *P. hysterophorus* should be taught to farmers, pastoralists students and the general public so that they can recognize the invasive as seedlings, removing them at an early growth stage and, thus, preventing large-scale invasion.

REFERENCES

Adamson, D., & Bray, S. (1999). *The economic benefit from investing in insect biological control of parthenium weed (Parthenium hysterophorus)* (Msc Thesis). The University of Queensland, Australia.

Adkins, S., & Shabbir, A. (2014). Biology, ecology and management of the invasive parthenium weed (*Parthenium hysterophorus* L.). *Pest Management Science*, 70(7), 1023-1029.

Adkins, S. W., Shabbir, A., & Dhileepan, K. (2018). *Parthenium Weed: Biology, Ecology and Management* (Vol. 11). Boston, United State of America: Centre for Agriculture and Bioscience Internation.

Akter, A., & Zuberi, M. (2009). Invasive alien species in northern Bangladesh: Identification, inventory and impacts. *International Journal of Biodiversity and Conservation*, 1(5), 129-134.

Al-Daihan, S., & Bhat, R. S. (2012). Antibacterial activities of extracts of leaf, fruit, seed and bark of Phoenix dactylifera. *African Journal of Biotechnology*, 11(42), 10021-10025.

Ali, S., & Khan, I. A. (2017). Management of *Parthenium hysterophorus* using suppressive plants. *Malaysian Journal of Science,* 36(2), 75-84.

Anjum, T., Bajwa, R., & Javaid, A. (2005). Biological control of *Parthenium hysterophorus*: Effect of *Imperata cylindrica* on distribution, germination and seedling growth of *Parthenium hysterophorus* L. *International Journal of Agriculture and Biology,* 7(3), 448-450.

Ayele, S., Nigatu, L., Tana, T., & Adkins, S. W. (2013). Impact of parthenium weed (*Parthenium hysterophorus* L.) on the above-ground and soil seed bank communities of rangelands in Southeast Ethiopia. *International Research Journal of Agricultural Science and Soil Science,* 3(7), 262-274.

Ayyanar, M., & Ignacimuthu, S. (2008). Pharmacological action of *Cassia auriculata* L. and *Cissus quadrangularis*. *Journal of Pharmacology and Toxicology,* 3(3), 213-221.

Badjie, M., Yaffa, S., Sawaneh, M., & Bah, A. (2019). Effects of climate variability on household food availability among rural farmers in central river region-south of the Gambia. *Scientific Report,* 5(17), 1-9.

Bajwa, A. A., Farooq, M., Nawaz, A., Yadav, L., Chauhan, B. S., & Adkins, S. (2019). Impact of invasive plant species on the livelihoods of farming households: Evidence from *Parthenium hysterophorus* invasion in rural Punjab, Pakistan. *Biological Invasions,* 21(11), 3285-3304.

Bangi, S., Lal, E. P., Bangi, S., & Sattigeri, U. (2014). Effect of herbicides on weed control efficiency (WCE) and yield attributes in brinjal (*Solanum melongena* L.). *Journal of Agriculture and Veterinary Science,* 7(6), 59-65.

Baryakabonaa, S., & Mwine, J. (2017). Evaluation of the efficacy of crude extracts of tick berry *Lantana camara* and mexican marigold *Tagetes manuta* Against cabbage aphids *Brevicoryne brassicae*. *American Scientific Research Journal for Engineering, Technology, and Sciences,* 5(2), 1-13.

Batish, D., Singh, H., Kohli, R., Kaur, S., Saxena, D., & Yadav, S. (2007). Assessment of parthenin against some weeds. *Zeitschrift für Naturforschung,* 4(1), 367-372.

Batish, D. R., Singh, H. P., Kohli, R. K., Johar, V., & Yadav, S. (2004). Management of invasive exotic weeds requires community participation1. *Weed Technology,* 18(1), 1445-1449.

Bergius, M., Benjaminsen, T. A., & Widgren, M. (2018). Green economy, Scandinavian investments and agricultural modernization in Tanzania. *The Journal of Peasant Studies,* 45(4), 825-852.

Blum, U. (1996). Allelopathic interactions involving phenolic acids. *Journal of Nematology,* 28(3), 259-270.

Boršić, I., Ješovnik, A., Mihinjač, T., Kutleša, P., Slivar, S., Cigrovski, M., … Desnica, S. (2018). Invasive alien species of union concern (Regulation 1143/2014) in Croatia. *Malaysian Journal of Science,* 27(2), 357-398.

Carson, A. (1979). Weed control in rainfed cotton in Northern Ghana. *International Journal of Agricultural Research, Innovation and Technology*, 25(4), 420-424.

Chaudhari, S. V., Gade, P. L., Tanpure, V. S., & Chaudhari, P. S. (2012). Ecofriendly control of carrot weed *Parthenium hysterophorus* using mexican beetle *Zygogramma bicolorata* around Rahuri City (Maharashtra-India). *International Journal of Innovative Research and Development,* 1(4), 238-245.

Chingwaru, W., Vidmar, J., & Kapewangolo, P. T. (2015). The potential of sub-saharan african plants in the management of human immunodeficiency virus infections: A review. *Phytotherapy Research,* 29(10), 1452-1487.

Chippendale, J., & Panetta, F. (1994). The cost of parthenium weed to the Queensland cattle industry. *Plant Protection Quarterly*, 9(2), 73-76.

Cock, M., & Holloway, J. (1982). The history of, and prospects for, the biological control of *Chromolaena odorata* (Compositae) by *Pareuchaetes pseudoinsulata* Rego Barros and allies (Lepidoptera: Arctiidae). *Bulletin of Entomological Research*, 72(2), 193-205.

Cohen, O., Gamliel, A., Katan, J., Kurzbaum, E., Riov, J., & Bar, P. (2018). Controlling the seed bank of the invasive plant *Acacia saligna:* Comparison of the efficacy of prescribed burning, soil solarization, and their combination. *Biological Invasions,* 20(10), 2875-2887.

Copeland, R. S., Wharton, R. A., Luke, Q., De-Meyer, M., Lux, S., Zenz, N., ... Okumu, M. (2006). Geographic distribution, host fruit, and parasitoids of African fruit fly pests *Ceratitis anonae, Ceratitis cosyra, Ceratitis fasciventris,* and *Ceratitis rosa* (Diptera: Tephritidae) in Kenya. *Annals of the Entomological Society of America,* 99(2), 261-278.

Datta, M. K., Kundu, C. K., Kundu, A., Hembram, J., Bera, P. S., & Biswas, U. (2018). Bio-efficacy of 2, 4-D ethyl ester 80% EC in non-crop area. *International Journal of Chemical Studies,* 6(1), 391-393.

Dhileepan, K. (2001). Effectiveness of introduced biocontrol insects on the weed *Parthenium hysterophorus* (Asteraceae) in Australia. *Bulletin of Entomological Research*, 91(3), 167-176.

Dhileepan, K. (2007). Biological control of parthenium (*Parthenium hysterophorus*) in Australian rangeland translates to improved grass production. *Weed Science*, 55(5), 497-501. DOI: https://doi.org/10.1614/WS-07-045.1.

Dhileepan, K., Callander, J., Shi, B., & Osunkoya, O. O. (2018). Biological control of parthenium (*Parthenium hysterophorus*): The Australian experience. *Biocontrol Science and Technology,* 28(10), 970-988.

Dhileepan, K., Setter, S., & Mcfadyen, R. (2000). Response of the weed *Parthenium hysterophorus* (Asteraceae) to defoliation by the introduced biocontrol agent *Zygogramma bicolorata* (Coleoptera: Chrysomelidae). *Biological Control,* 19(1), 9-16.

Dhileepan, K., & Strathie, L. (2009). *Parthenium hysterophorus* L.(Asteraceae). Biological control of tropical weeds using arthropods. *Canadian Journal of Botany,* 50(8), 274-318.

Dhileepan, K., & Senaratne, K. (2009). How widespread is *Parthenium hysterophorus* and its biological control agent *Zygogramma bicolorata* in South Asia? *Weed Research,* 49(6), 557-562.

Dogra, K. S., Sood, S. K., & Sharma, R. (2011). Distribution, biology and ecology of *Parthenium hysterophorus* L.(congress grass) an invasive species in the north-western Indian Himalaya (Himachal Pradesh). *African Journal of Plant Science,* 5(11), 682-687.

Lemaire, F. (1990). The flora and phytogeography of the evergreen forests of Malawi lowland forests.*Canadian Journal of Botany, 60*(1/2), 9-71.

Evans, H. C. (1997). *Parthenium hysterophorus*. a review of its weed status and the possibilities for biological control. *Biocontrol News and Information,* 18(89N-98N), 10-20.

Falster, D. S., & Westoby, M. (2003). Plant height and evolutionary games. *Trends in Ecology and Evolution,* 18(7), 337-343. DOI: https://doi.org/10.1016/S0169-5347(03)00061-2.

Foxcroft, L., Lotter, W., Runyoro, V., & Mattay, P. (2006). A review of the importance of invasive alien plants in the Ngorongoro conservation area and Serengeti national park.

African Journal of Ecology, 44(3), 404-406. DOI: https://doi.org/10.1111/j.1365-2028.2006.00607.x.

Funk, C., Peterson, P., Landsfeld, M., Pedreros, D., Verdin, J., Shukla, S., … Hoell, A. (2015). The climate hazards infrared precipitation with stations. A new environmental record for monitoring extremes. *Scientific Data*, 2(3), 15-66.

Geldenhuys, C. (1992). Richness, composition and relationships of the floras of selected forests in southern Africa. *Bothalia,* 22(2), 205-233.

Gnanavel, I., & Natarajan, S. (2013). *Parthenium hysterophorus* L. A major threat to natural and agro eco-systems in India. *International Journal of Agriculture, Environment and Biotechnology,* 6(2), 261-269.

Gupta, O., & Sharma, J. (1977). *Parthenium hysterophorus* menace in India and possible control measures. *Plant Protection Bulletin*, 25(3), 112-117.

Gupta, S., Sharma, S. B., Prabhu, K. M., & Bansal, S. K. (2009). Protective role of *Cassia auriculata* leaf extract on hyperglycemia-induced oxidative stress and its safety evaluation. *Canadian Journal of Botany,* 45(10), 1-60.

Guyana, P., & Paraguay, S. (2014). *Parthenium hysterophorus* L. Asteraceae – Parthenium weed. *Current Science,* 44(3), 474-478.

Hammerton, J. (1981). Weed problems and weed control in the commonwealth Caribbean. *International Journal of Pest Management,* 27(3), 379-387.

Hassan, G., Rashid, H., Amin, A., Khan, I., & Shehzad, N. (2018). Allelopathic effect of *Parthenium hysterophorus* on germination and growth of some important crops and weeds of economic importance. *Planta Daninha,* 36(1), 1-11. DOI: http://dx.doi.org/10.1590/s0100-83582018360100132

Hiscox, J., & Israelstam, G. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany,* 57(12), 1332-1334. DOI: https://doi.org/10.1139/b79-163.

Hundessa, N. (2016). Socio-economic impacts of *Parthenium hysterophorus* L. in east Shewa and west Arsi zones of Ethiopia. *International Journal of Agricultural Research, Innovation and Technology,* 6(2), 5-11. DOI: https://doi.org/10.3329/ijarit.v6i2.31696.

Jalali, M., Gheysari, H., Azizi, M., Zahedi, S., & Moosavi, S. (2013). Allelopathic potential of common *mallow* (*Malva sylvestris*) on the germination and the initial growth of blanket flower, plumed cockscomb and sweet william. *International Journal of Agriculture and Crop Sciences,* 5(15), 1638-1641.

Javaid, A., Anjum, T., & Bajwa, R. (2005). Biological control of *Parthenium hysterophorus*: Allelopathic effect of *Desmostachya bipinnata* on distribution and early seedling growth of *Parthenium hysterophorus* L. *International Journal of Biology and Biotechnology,* 2(2), 459-463.

Javaid, A., Shafique, S., Kanwal, Q., & Shafique, S. (2010). Herbicidal activity of flavonoids of mango leaves against *Parthenium hysterophorus* L. *Natural Product Research,* 24(19), 1865-1875.

Jayanth, K. (1987). Introduction and establishment of *Zygogramma bicolorata* on *Parthenium hysterophorus* at Bangalore, India. *Current Science,* 56(7), 310-311.

Jayanth, K., & Bali, G. (1993). Biological studies on *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), a potential biocontrol agent of *Parthenium hysterophorus* L.(Asteraceae). *Journal of Biological Control,* 7(2), 93-98.

Jayanth, K., & Bali, G. (1995). Effect of soil moisture on pupation and adult emergence of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae). *Journal of Entomological Research,* 19(2), 183-185.

Joshi, S. (1991). Interference effects of *Cassia uniflora* Mill on *Parthenium hysterophorus* L. *Plant and Soil,* 132(2), 213-218.

Kapoor, R. (2012). Awareness related survey of an invasive alien weed, *Parthenium hysterophorus* L. in Gautam Budh Nagar district, Uttar Pradesh, India. *Journal of Agricultural Technology,* 8(3), 1129-1140.

Karim, S., Nurzafirah, Z., & Norhafizah, M. (2017). Weed seed bank of Parthenium weed (*Parthenium hysterophorus* L.) in Batang Kali, Selangor, Malaysia. *Pertanika Journal of Tropical Agricultural Science,* 40(4), 1-15.

Kaur, M., Aggarwal, N. K., Kumar, V., & Dhiman, R. (2014). Effects and management of *Parthenium hysterophorus*: A weed of global significance. *International Scholarly Research Notices*, 2014(1), 1-20.

Khaket, T. P., Aggarwal, H., Jodha, D., Dhanda, S., & Singh, J. (2015). *Parthenium hysterophorus* in current scenario: A toxic weed with industrial, agricultural and medicinal applications. *Journal of Plant Science,* 10(2), 42-53.

Khalaj, M., Amiri, M., & Azimi, M. (2013). Allelopathy: Physiological and sustainable agriculture important aspects. *International Journal of Agronomy and Plant Production,* 4(5), 950-962.

Khaliq, A., Matloob, A., Tanveer, A., Areeb, A., Aslam, F., & Abbas, N. (2011). Reduced doses of a sulfonylurea herbicide for weed management in wheat fields of Punjab, Pakistan. *Chilean Journal of Agricultural Research,* 71(3), 424-425.

Khan, O'Donnell, C., Shabbir, A., & Adkins, S. W. (2010). Competitive displacement of parthenium weed with beneficial native and introduced pasture plants in central Queensland, Australia. *Weed Research*, 6(3), 31-134.

Khan, H., Marwat, K. B., Hassan, G., & Khan, M. A. (2013a). Socio-economic impacts of parthenium (*Parthenium hysterophorus* L.) in Peshawar valley, Pakistan. *Pakistan Journal of Weed Science Research,* 19(3), 60-72.

Khan, N., O'donnell, C., George, D., & Adkins, S. (2013b). Suppressive ability of selected fodder plants on the growth of *Parthenium hysterophorus*. *Weed Research,* 53(1), 61-68. DOI: https://doi.org/10.1016/j.jenvman.2019.06.051.

Kigel, J. (2017). Seed germination in arid and semiarid regions. *Routledge,* 5(2), 645-699.

Kija, B., Mweya, C. N., Mwita, M., & Fumagwa, R. (2013). Prediction of suitable habitat for potential invasive plant species *Parthenium hysterophorus* in Tanzania: A short communication. *International Journal of Ecosystem,* 3(4), 82-86. DOI: https://doi.org/10.5923/j.ije.20130304.04.

Kilewa, R., & Rashid, A. (2014). Distribution of invasive weed *Parthenium hysterophorus* in natural and agro-ecosystems in Arusha Tanzania. *International Journal of Science and Research,* 3(12), 1-4.

Kilkenny, M. F., Purvis, T., Werner, M., Reyneke, M., Czerenkowski, J., & Cadilhac, D. A. (2016). Improving stroke knowledge through a 'volunteer-led'community education program in Australia. *Preventive Medicine,* 86(2016), 1-5.

Kohli, R. K., Batish, D. R., Singh, H., & Dogra, K. S. (2006). Status, invasiveness and environmental threats of three tropical American invasive weeds (*Parthenium hysterophorus* L., *Ageratum conyzoides* L., *Lantana camara* L.) in India. *Biological Invasions,* 8(7), 1501-1510.

Kumar, S. (2009). Biological control of *Parthenium hystrophorus* in India: Status and prospects. *Indian Journal of Weed Science,* 41(1-2), 1-18.

Kundu, A., Kundu, C. K., Hedayetullah, M., Datta, M. K., Bairagya, M. D., & Biswas, U. (2018). Performance of 2, 4-D Dimethyl amine 50% SL on weed control of non-crop area under new alluvial zone of west Bengal. *Journal of Pharmacognosy and Phytochemistry,* 7(1), 1187-1190.

Louda, S. M., Kendall, D., Connor, J., & Simberloff, D. (1997). Ecological effects of an insect introduced for the biological control of weeds. *Science,* 277(5329), 1088-1090.

Mahmoud, T., Gairola, S., & El-Keblawy, A. (2015). *Parthenium hysterophorus* and *Bidens pilosa*, two new records to the invasive weed flora of the United Arab Emirates. *Journal on New Biological Reports,* 4(1), 26-32.

Mahugija, J. A. (2013). Status and distributions of pesticides buried at five sites in Arusha and Mbeya regions, Tanzania. *African Journal of Pure and Applied Chemistry,* 7(2), (382-393.

Manjunath, T. (2010). *Biological control of the wasteland weed, Parthenium hysterophorus, in India – An overview.* 3rd International Conference on Parthenium organized by and at Indian Agricultural Research Institute, India.

Mcconnachie, A. J. (2015). Host range and risk assessment of *Zygogramma bicolorata*, a defoliating agent released in South Africa for the biological control of *Parthenium hysterophorus*. *Biocontrol Science and Technology*, 25(9), 975-991.

Mcfadyen, R. C. (1992). Biological control against *Parthenium hystrophorus* weed in Australia. *Crop Protection,* 11(5), 400-407.

Minnaar, P., Jolly, N., Paulsen, V., Du-Plessis, H., & Vander, M. (2017). *Schizosaccharomyces pombe* and S*accharomyces cerevisiae* yeasts in sequential fermentations: Effect on phenolic acids of fermented Kei-apple (*Dovyalis caffra L.*) juice. *International Journal of Food Microbiology,* 257(232-237.

Mishra, J., & Bhan, V. (1996). Chemical control of carrot grass (*Parthenium hysterophorus*) and associated weeds in soybean (*Glycine max*). *Springer Plus*, 5(2), 1-18.

Moshi, M. J., & Mbwambo, Z. H. (2002). Experience of Tanzanian traditional healers in the management of non-insulin dependent diabetes mellitus. *Pharmaceutical Biology,* 40(7), 552-560. DOI: https://doi.org/10.1076/phbi.40.7.552.14691.

Msafiri, C. J., Mokiti, T., & Patrick, A. N. (2013). Allelopathic effects of *Parthenium hysterophorus* on seed germination, seedling growth, fresh and dry mass production of *Alysicurpus glumaceae* and *Chloris gayana*. *American Journal of Research Communication*, 1(11), 190-205.

Mussa, M., Teka, H., & Mesfin, Y. (2017). Land use/cover change analysis and local community perception towards land cover change in the lowland of Bale rangelands, Southeast Ethiopia. *International Journal of Biodiversity and Conservation*, 9(12), 363-372.

Narasimhan, T., Ananth, M., Swamy, M. N., Babu, M. R., Mangala, A., & Rao, P. S. (1977). Toxicity of *Parthenium hysterophorus* L. to cattle and buffaloes. *Experientia*, 33(10), 1358-1359.

Navie, S., Mcfadyen, R., Panetta, F., & Adkins, S. (1996). The biology of Australian weeds. 27. *Parthenium hysterophorus* L. *Plant Protection Quarterly,* 11(2), 76-88.

Navie, S., Panetta, F., Mcfadyen, R., & Adkins, S. (1998). Behaviour of buried and surface-sown seeds of *Parthenium hysterophorus*. *Weed Research,* 38(5), 335-341.

Ngondya, I. B., Munishi, L. K., Treydte, A. C., & Ndakidemi, P. A. (2016). A nature-based approach for managing the invasive weed species *Gutenbergia cordifolia* for sustainable rangeland management. *Springer Plus*, 5(1), 17-87.

Nille, G. C., & Reddy, K. (2015). A phytopharmacological review of plant-*Cassia auriculata. International Journal of Pharmaceutical and Biological Archive,* 6(1), 1-9.

Noor, M., & Khan, M. (1994). Allelopathic potential of *Albizia samans* Merr. *Pakistan Journal of Botany,* 26 (1), (139-139.

Nyasembe, V. O., Cheseto, X., Kaplan, F., Foster, W. A., Teal, P. E., Tumlinson., … Torto, B. (2015). The invasive American weed *Parthenium hysterophorus* can negatively impact malaria control in Africa. *Plos One,* 10(9), 13-78.

Ojija, F., Arnold, S. E., & Treydte, A. C. (2019). Bio-herbicide potential of naturalised *Desmodium uncinatum* crude leaf extract against the invasive plant species *Parthenium hysterophorus*. *Biological Invasions,* 9(2), 1-13. DOI: https://doi.org/10.1007/s10530-019-02075-w.

Omotayo, A. O., Ncama, K., & Aremu, A. O. (2018). Potentials of the underutilized kei-apple (*Dovyalis caffra* L.): Beyond its nutritional and functional value. *South Africa Journal of Botany,* 5(2), 1-5. DOI: https://doi.org/10.20944/preprints201812.0094.v1.

Parker, I. M., & Reichard, S. H. (1998). Critical issues in invasion biology for conservation science. *Springer Plus*, 10(2), 283-305.

Patel, S. (2011). Harmful and beneficial aspects of *Parthenium hysterophorus*: An update. *Biotech,* 1(1), 1-9.

Prins, A., & Verkaar, H. (1992). Defoliation: Do physiological and morphological responses lead to (over) compensation? *Scholastic*, 10(5), 13-31.

Raj, J. Y., Peter, M. P. J., & Joy, V. (2012). Chemical compounds investigation of *Cassia auriculata* seeds: A potential folklore medicinal plant. *Asian Journal of Plant Science and Resources*, 2(2), 187-192.

Ray, P., & Gour, H. (2012). Integrated management of *Parthenium hysterophorus* L.(Asteraceae): A weed of worldwide significance. *Indian Society of Mycology and Plant Pathology,* 5(605-632.

Raza, A., Kamran, M., Safdar, M. E., Ali, H. H., Abbas, T., Asif, M., … Rehman, A. (2019). Management tactics for handling *Parthenium hysterophorus* in non-native environment through phytotoxic compounds of local species. *International Journal of Agriculture and Biology,* 21(1), 215-222.

Richardson, D. M., Holmes, P. M., Esler, K. J., Galatowitsch, S. M., Stromberg, J. C., Kirkman., … Hobbs, R. J. (2007). Riparian vegetation: Degradation, alien plant invasions, and restoration prospects. *Diversity and Distributions*, 13(1), 126-139.

Rwomushana, I., Godwin, J., Constantine, K., Makale, F., Nunda, W., Day., … Gonzalez-Moreno, P. (2019). Parthenium: Impacts and coping strategies in Central West Asia. *Asian Journal of Plant Sciences,* 9(2), 22-37.

Saha, D., Marble, C., Stamps, R. H., Steed, S., & Boyd, N. S. (2018). Biology and management of ragweed parthenium (*Parthenium hysterophorous* L.) in ornamental crop production. *Pakistan Journal of Botany,* 5(2), 1-6.

Saifuddin, M., Hossain, A., & Normaniza, O. (2010). Impacts of shading on flower formation and longevity, leaf chlorophyll and growth of *Bougainvillea glabra*. *Asian Journal of Plant Sciences*, 9(1), 20-33.

Schmelzer, G. H. (2008). Factors affecting the demand of medicinal plants. *Scientific Data*, 11(3), 1-18.

Seta, T., Assefa, A., Mesfin, F., & Balcha, A. (2013). Distribution status and the impact of parthenium weed (*Parthenium hysterophorus* L.) at Gedeo zone (Southern Ethiopia). *Asian Journal of Agriculture Research*, 8(4), 386-397.

Shabbir, Dhileepan, K., Khan, N., & Adkins, S. (2014). Weed pathogen interactions and elevated C_{O2}: Growth changes in favour of the biological control agent. *Weed Research,* 54(3), 217-222.

Shabbir, A. (2014). Chemical control of *Parthenium hysterophorus* L. *Pakistan Journal of Weed Science Research,* 20(1), 1-11.

Shabbir, A., Bajwa, A. A., Dhileepan, K., Zalucki, M., Khan, N., & Adkins, S. (2018). Integrated use of biological approaches provides effective control of *Parthenium hystrophorus*. *Archieves of Agronomy and Soil Science*, 64(13), 1861-1878.

Shabbir, A., & Bajwa, R. (2006). Distribution of parthenium weed (*Parthenium hysterophorus* L.), an alien invasive weed species threatening the biodiversity of Islamabad. *Weed Biology and Management,* 6(2), 89-95. DOI: https://doi.org/10.1111/j.1445-6664.2006.00202.x.

Shabbir, A., Dhileepan, K., O'Donnell, C., & Adkins, S. W. (2013). Complementing biological control with plant suppression: Implications for improved management of parthenium weed (*Parthenium hysterophorus* L.). *Biological Control,* 64(3), 270-275. DOI: https://doi.org/10.1016/j.biocontrol.2012.11.014.

Shabbir, A., Dhileepan, K., Zalucki, M. P., O'Donnell, C., Khan, N., Hanif, Z., … Adkins, S. W. (2015). The combined effect of biological control with plant competition on the management of parthenium weed (*Parthenium hysterophorus* L.). *Pakistan Journal of Botany,* 47(SI), 157-159.

Shabbir, A., Sadia, S., & Mujahid, I. (2016). Biocontrol efficiency of *Zygogramma bicolorata* at different growth stages of *Parthenium hysterophorus*. *Indian Journal of Weed Science,* 48(4), 460-463.

Shrestha, B., Shabbir, A., & Adkins, S. (2015). *Parthenium hysterophorus* in Nepal: A review of its weed status and possibilities for management. *Weed Research,* 55(2), 132-144. DOI: https://doi.org/10.1111/wre.12133.

Shrestha, B. B., Shrestha, U. B., Sharma, K. P., Thapa-Parajuli, R. B., Devkota, A., & Siwakoti, M. (2019). Community perception and prioritization of invasive alien plants in

Chitwan-Annapurna Landscape, Nepal. *Journal of Environmental Management,* 229(3), 38-47.

Simberloff, D. (2013). *Invasive species: what everyone needs to know*: Oxford University, England.

Singh, S., Gill, P. K., Dhaliwal, H., & Kumar, V. (2017). Life cycle and effectiveness of *Zygogramma bicolorata* Pallister (Chrysomelidae: Coleoptera) on *Parthenium hysterophorus* eradication. *Journal of Global Agriculture and Ecology,* 7(2), 60-65.

Singh, S., Yadav, A., Balyan, R. S., Malik, R. K., & Singh, M. (2004). Control of ragweed parthenium (*Parthenium hysterophorus*) and associated weeds. *Weed Technology,* 18(3), 658-664.

Smith, H. S., & Debach, P. (1942). The measurement of the effect of entomophagous insects on population densities of their hosts. *Journal of Economic Entomology,* 35(6), 845-849.

Soltys, D., Krasuska, U., Bogatek, R., & Gniazdowska, A. (2013). Allelochemicals as bioherbicides present and perspectives. *IntechOpen*, 10(2), 1-8.

Stone, C. M., Witt, A. B., Walsh, G. C., Foster, W. A., & Murphy, S. T. (2018). Would the control of invasive alien plants reduce malaria transmission? A review. *Parasites & Vectors*, 11(1), 76-100.

Strathie, L. (2015a). Managing the invasive alien plant *Parthenium hysterophorus* in South Africa. *Grassroots,* 15(2), 15-21.

Strathie, L. (2015b). Managing the invasive alien plant *Parthenium hysterophorus* in South Africa. *Grassroots,* 15(2), 15-21.

Tamado, T., Ohlander, L., & Milberg, P. (2002). Interference by the weed *Parthenium hysterophorus* L. with grain sorghum: Influence of weed density and duration of competition. *International Journal of Pest Management,* 48(3), 183-188.

Tanveer, A., Khaliq, A., Ali, H. H., Mahajan, G., & Chauhan, B. S. (2015). Interference and management of *Parthenium hysterophorus*: The world's most important invasive weed. *Crop Protection,* 68(6), 49-59. DOI: org/10.1016/j.cropro.2014.11.005.

Taye, T., Einhorn, G., Gossmann, M., Büttner, C., & Metz, R. (2004). The potential of rust as a biological control of *Parthenium hystrophorus* in Ethiopia. *Pest Management Journal of Ethiopia,* 8(2), 83-95.

Tesfu, F., & Emana, G. (2013). Evaluation of *Parthenium hysterophorus* L. powder against *Callosobruchus chinensis* L.(Coleoptera: Bruchidae) on chickpea under laboratory conditions. *African Journal of Agriculture Research*, 8(5405-5410.

Thakur, D. (1994). Weed management intercropping in intercropping systems based on maize under rainfed mid hill conditions. *Indian Journal of Agronomy,* 39(2), 203-206.

Tibesigwa, B., Siikamäki, J., Lokina, R., & Alvsilver, J. (2019). Naturally available wild pollination services have economic value for nature dependent smallholder crop farms in Tanzania. *Scientific Reports,* 9(1), 34-39.

Wabuyele, E., Lusweti, A., Bisikwa, J., Kyenune, G., Clark, K., Lotter, W. D., … Wondi, M. (2014). A roadside survey of the invasive weed *Parthenium hysterophorus* (Asteraceae) in East Africa. *Journal of East African Natural History,* 103(1), 49-58. DOI: https://doi.org//10.2982/028.103.0105

Wagner, V., Antunes, P. M., Irvine, M., & Nelson, C. R. (2017). Herbicide usage for invasive non-native plant management in wildland areas of North America. *Journal of Applied Ecology,* 54(1), 198-204. DOI: https://doi.org/10.1111/1365-2664.12711.

Wakjira, M., Berecha, G., & Tulu, S. (2009). Allelopathic effects of an invasive alien weed *Parthenium hysterophorus* L. compost on lettuce germination and growth. *African Journal of Agricultural Research,* 4(11), 1325-1330.

Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A., & Wright, I. J. (2002). Plant ecological strategies: Some leading dimensions of variation between species. *Annual Review of Ecology and Systematics,* 33(1), 125-159.

Witt, A. (2010). Impacts of invasive plants and their sustainable management in agro-ecosystems in Africa: A review. *International Journal of Agricultural Research, Innovation and Technology,* 3(2), 1102-1109.

Worku, M. (2010). Prevalence and distribution survey of an invasive alien weed (*Parthenium hysterophorus* L.) in Sheka zone, southwestern Ethiopia. *African Journal of Agricultural Research*, 5(9), 922-927.

Yadav, N., Saha, P., Jabeen, S., Kumari, S., Verma, S., Singh, B., … Sinha, M. (2010). Effect of methanolic extract of *Parthenium hysterophorus* on haematological parameters in wistar albino rat. *The Bioscan International Journal of Life Sciences*, 2(4), 357-363.

APPENDICES

Appendix 1: Questionnaire to farmers

A. Enumerator………………

B. Household Identification and Demography

1. Division………………….
2. Ward…………………
3. Village………………….
4. Name of HH Head……………………
5. Year born of household head ……………….
6. Gender of household head
    a. Male b. Female
7. Highest education level of household head
    a. No formal education
    b. Some primary school
    c. Completed primary school
    d. Some secondary school
    e. Completed secondary school
    f. Post-secondary qualifications
    g. Some university
    h. Completed university
    i. Post graduate
8. Was the household head born in this village?
    a. Yes b. No

9. If NO, how long has the respondent lived in the village?
10. How many family members are there in total in your household?.............

C. Questions about *P. hysterophorus*

1. Have you ever seen this plant?
    a. Yes b. No
2. If YES, when did you see the plant for the first time in your village?
    a. Between 2 and 5 years

b. Between 6 and 10 years
c. More than 10 years

3. Where do you normally see it?
    a. Farm
    b. Pasture
    c. Along roads
    d. Other
4. Have you experienced the presence of P. hysterophorus on your farm or surrounding areas?
    a. Yes b. No

5. How big is your farm?
    a. 1 acre
    b. 2-5 acres
    c. 6-10 acres
    d. More than 10 acres
6. Have you noticed any benefit to your crops due to presence of this weed?
    a. Yes b. No
7. How has this weed affected farming activities?
    a. Highly
    b. Little
    c. Fluctuates over time
    d. Have not affected
8. Which crops do you grow?
    a. Cereals
    b. Vegetables
    c. Both
    d. Other
9. Do you have livestock?
    a. Yes b. No
10. If YES, what type?
    a. Cattle
    b. Goat
    c. Sheep

11. Does Parthenium affect your livestock?
    a. Yes b. No

12. In what extent is the weed growing?
    a. Dense b. Sparse
13. Is there any problem for you from this weed in farming?
    a. Yes b. No
14. If YES, mention…………………………….
15. Does it decrease the productivity of your crops?
    a. Yes b. No
16. If YES, by how much?
    a. Reduces yield by less than quarter
    b. Reduces yield by quarter
    c. Reduces yield by half
    d. Reduces yield by three quarters
    e. Reduces yield by more than three quarters
17. Which crop(s) is highly reduced?...............................

18. Have you used this weed for any purpose?
    a. Yes b. No
19. If YES, how do you use it?...........................
20. Did you notice any benefits from its use?
    a. Yes b. No
21. What is your source of income?
    a. Farming
    b. Cattle grazing
    c. Other activities
22. At what level does *P. hysterophorus* affect your income
    a. Highly
    b. Little
    c. Fluctuates over time
    d. Have not affected
23. Have you tried any measure to control this weed?
    a. Yes b. No

24. If YES, what measure?...........................
25. How was the experience in particular control measures of the weed?................
26. Are you aware about biological control using beetle *Z. bicolorata*?
    a. Yes b. No

Appendix 2: Questions to pastoralist

1. What type of field do your livestock's prefer to graze?
    a. Open rangeland
    b. Along roads
    c. On fields
    d. On the infested land
    e. Anywhere
2. What time of the year do your livestock graze on Parthenium?
    a. Wet
    b. Dry
    c. Both
3. Have you ever noticed any health problems to your livestock's?
    a. Yes b. No
4. If YES, mention health problems…………………
5. Did you follow any medical treatments to your livestock's for the problems?
    a. Yes b. No
6. If YES, how much money spent on treatment?
    a. 10000-30000
    b. 30000-50000
    c. Beyond 50000
7. Is there any difference in milk quality after grazing in this weed field?
    a. Yes b. No
8. If YES, what are they?.......................
9. Is there observed difference in milk quantity after grazing in this weed field?
    a. Yes b. No
10. If YES, what are they?....................
11. Is there any change in the fertility of your livestock's?
    a. Yes b. No
12. If yes, What changes?...........................
13. Have you experienced any problem to your health?
    a. Yes b. No
14. If YES, which problem(s)?..........................

15. Did you follow any medical treatment in regard to these problems?
    a. Yes b. No
16. If YES, how much money spent on treatment?
    a. 10000-30000
    b. 30000-50000
    c. Beyond 50000
17. Have you tried any measure to control this weed?
    b. Yes b. No
18. If YES, what measure?...........................
19. How was the experience in particular control measures of the weed?................
20. Are you aware about biological control using beetle *Z. bicolorata*?
    b. Yes b. No

Appendix 3: Introduction letter from Nelson Mandela African Institution of Science and Technology

THE NELSON MANDELA
AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY
(NM-AIST)

School of Life Sciences and Bioengineering

Direct Line: +255 272555070
Fax: +255 272555071
E-mail: deanlsbe@nm-aist.ac.tz

Tengeru
P.O. Box 447
Arusha, TANZANIA
Website: www.nm- aist.ac.tz

OUR Ref.No. NM-AIST/M.530/T.17/05

Date: 11th January, 2019

To Whom It May Concern

Dear Sir/Madam,

RE: INTRODUCTION TO MS. WARDA KANAGWA

Kindly refer to the above heading.

I wish to introduce Ms. Warda Kanagwa with Registration No. NM-AIST/M.530/T.17, a Master's student at Nelson Mandela African Institution of Science and Technology in the School of Life Sciences and Bioengenering.

As part of the requirement for Master's degree, Ms. Warda is undertaking a research with title ***"Effectiveness of Biological Control and Socio-economic Impacts of the Invasive Parthenium Hysterophorus in Arusha, Tanzania".***

In order to accomplish her research objectives, she would like to collect some information from your organization/region. The information to be collected will be used for research purposes only and will give a picture of Effectiveness of Biological Control and Socio-economic Impacts of the Invasive Parthenium Hysterophorus in Arausha,Tanzania as it states in the research objectives.

It is my sincere hope you will assist the student in accomplishing her study.

Looking forward for your cooperation.

Sincerely,

Dr. Gabriel Shirima
Ag. Dean

Appendix 4: Research clearance letter from Monduli district council to conduct research at Sepeko ward

MONDULI DISTRICT COUNCIL

ARUSHA REGION
All Correspondences to be addressed to
The District Executive Director,
Tel. No. +255 - 27- 2538006 G.L
+255 - 27- 2538005 D.L
Fax No. +255 - 27- 2538136/361
E-mail: ded@mondulidistrict.go.tz
In reply please quote:

COUNCIL HALL,
P.O. BOX 1,
MONDULI.

Ref. No. HW/MON/R5/1 VOL II/357

18th March, 2019

Principal,
The Nelson Mandela African Institution of Science and Technology,
P. o. Box 447,
TENGERU.

RE: A REQUEST FOR RESEARCH CLEARANCE

Reference is made to your letter with reference number NM-AIST/M.17/05 dated 11th January, 2019 concerning the above subject matter.

This is to confirm that the permit has been granted to ***Ms. Warda Kanagwa*** as requested.

The title of the research is "***Effectiveness of Biological Control and Sociao-economic Impacts of the Invasive Parthenium Hysterophorus in Bioengineering"*** A Case of Monduli District Council.

This research will take place **Sepeko** Ward beginning **20th March, 2019** to **10th April, 2019**

It our expectation that the said data collection will let towards achieving her objective goal. In due course, there will be no financial implication to the Council.

Elifitia Pallangyo.
For: DISTRICT EXECUTIVE DIRECTOR,
MONDULI.

Mkurugenzi Mtendaji
Halmashauri ya Wilaya Monduli

Copy to:

- **WEO – Sepeko -** You are requested to accept ***Ms . Warda Kanagwa*** and assign her work during her practical
- Ms. Warda Kanagwa ,
  Student of The Nelson Mandela African Institution of Science and Technology.

Appendix 5: Research clearance letter from Arusha city council to conduct research at Olasiti ward

UNITED REPUBLIC OF TANZANIA
PRIME MINISTER'S OFFICE
REGIONAL ADMINISTRATION AND LOCAL GOVERNMENTS

ARUSHA CITY COUNCIL

All correspondences addressed to:
Phone: +255 27 2508073/2503494 (Director)
+255 27 2544330 (General)
Fax: +255 27 2505013

On reply please quote:
Ref. No. CD/R.30/32/

City Hall
P.O. Box 3013
ARUSHA, TANZANIA
e-mail: cd@arushacity.go.tz
Website: www.arushacity.go.tz

Date: **31st January, 2019**

Principal
Nelson Mandela African Institution of Science and Technology
P.O. Box 447
ARUSHA

Re: DATA COLLECTION REQUEST FOR MS WARDA KANAGWA

Reference is made to your letter with the above caption.

I would like to inform you that permission is granted to the above mentioned person to collect data titled ***"Effectiveness of Biological Control and Socio-economic Impacts of the Invasive Parthenium Hysterophorus in Arusha Tanzania"*** at **Olasiti Ward** within Arusha City Council.

However the data collection costs remains their responsibility due to financial constraints in our Council.

He should report to **WEO – Olasiti** with the copy of this letter.

Sarah Chupa
***For:* CITY DIRECTOR**
ARUSHA

For: CITY DIRECTOR
ARUSHA CITY COUNCIL

Copy : Ms Warda Kanagwa - For information and follow up
: WEO - Olasiti - Please accept and assist

RESEARCH OUTPUT

Journal paper

Effectiveness of *Zygogramma bicolorata* as a biocontrol agent against *Parthenium hysterophorus* in Arusha, Tanzania

[1*]Warda Kanagwa, [2] Ramadhan Kilewa, [1,3]Anna C. Treydte,

[1]*Nelson Mandela African Institution of Science and Technology, P. O. Box 447, Arusha, Tanzania*

[2]*Tropical Pesticides Research Institute, P. O. Box 3024 Arusha, Tanzania*

[3]*Hans Ruthenberg Institute, Agroecology in the Tropics and Subtropics, University of Hohenheim, Garbenstr. 13, 70599 Stuttgart, Germany.*

*Corresponding Author: kanagwaw@nm-aist.ac.tz

Abstract

Parthenium hysterophorus is an invasive weed that poses significant threats to crop production, biodiversity, human and animal health. Few experiments have been carried out to test the effectiveness of biological control agents such as the beetle *Zygogramma bicolorata* against this invasive. We released *Z. bicolorata* beetles as bio-agent to control *P. hysterophorus* at the Tropical Pesticides Research Institute in Arusha, Tanzania, across two seasons, from February – July 2019 (wet) and August 2019 – January 2020 (dry). Feeding by *Z. bicolorata* reduced *P. hysterophorus* leaves, flowers, height as well as biomass, both in the dry and wet season. During the wet season, 100% of all *P. hysterophorus* leaves were eaten already 28 days after the onset of the experiment, particularly under the highest beetle population treatment (30 individuals). *Parthenium hysterophorus* flower numbers were greatly reduced under high beetle populations (30 individuals) compared to the control treatment (0 individuals) during both the wet and dry season. *Parthenium hysterophorus* height was reduced by 87% and 90% during the wet and dry season, respectively when 30 beetles were released. Similarly, biomass was reduced by 90% and 87% during the wet and dry season, respectively. *Parthenium hysterophorus* responses to beetle treatments did not differ significantly across seasons but the development of both the invasive and its control, *Z. bicolorata,* was slightly delayed in the dry season. We conclude that *Z. bicolorata* can be used as bio-agent to manage *P. hysterophorus* in Tanzania, particularly when released in large numbers.

Keywords: Biological control, Beetles, Famine weed, Biomass, Eastern Africa

Introduction

Parthenium hysterophorus is an annual, herbaceous flowering plant native to the areas surrounding the Gulf of Mexico, Central America, southern North America, and West (Navie et al., 1996; Dogra et al., 2011). This weed has invaded 48 countries worldwide spreading over five continents and numerous islands (Adkins et al., 2018a). In Africa, *P. hysterophorus* has invaded more than ten countries including Somalia, Ethiopia, Kenya, Uganda, Tanzania, South Africa, Swaziland, Zimbabwe, and Madagascar (Wabuyele et al., 2014). In addition, climatic modeling predictions show that many parts of sub-Saharan Africa are at risk of *P. hysterophorus* invasion (Mcconnachie et al., 2011). In Tanzania for instance, the presence of *P. hysterophorus* was first recorded in 2010 in the northern part of the country (Arusha and Kilimanjaro regions; Kija et al., 2013; Wabuyele et al., 2014). The weed later spread rapidly to other regions such as Manyara, Geita, and Kagera (Kilewa and Rashid, 2014), affecting agricultural and pastoral. The species is estimated to have invaded about 10,000 ha of farms and grazing land in these three regions (Ramadhan, pers.comm., April 2018).

Parthenium hysterophorus can reduce maize and sorghum production by 40% and 95% (Kumar, 2009) and has caused a reduction of livestock forage production by 92% in Ethiopia (Adkins and Shabbir, 2014). This species poses serious threats to human and animal health, agricultural and pasture production, and biodiversity in the invaded areas around the globe (Seta et al., 2013). *Parthenium hysterophorus* is associated with human diseases such as allergic rhinitis, bronchitis, dermatitis, hay fever, allergic eczematous, and mental depression (Shrestha et al., 2015). Also, *P. hysterophorus* causes allergic reactions to cows, buffalo, and goats (Ayele et al., 2013). The weed also reduces the quality and quantity of milk and meat (Seta et al., 2013).

Several methods have been attempted by farmers and pastoralists to control the weed but many of those methods have faced limitations and led to adverse effects. Both physical and chemical methods are being used by farmers and pastoralists to control this weed, which can be effective on a small scale infestation but is not practical and economical in large areas that had been infested by *P. hysterophorus* (Adkins et al., 2018b). Furthermore, using herbicides can cause pollution and negatively impact non-targeted organisms (Ngondya et al., 2016).

The use of biological methods has recently been emphasized because it is sustainable, eco-friendly, and cost-effective over large areas (Adkins et al., 2018b). Various bio-agents have been used successfully such as *Pareuchaetes pseudoinsulata* Rego Barros and *Cecidochares connexa* Macquart, which had been prioritized as candidate agents in Nigeria and Ghana to suppress *Chromolaena odorata* (Cock and Holloway, 1982). *Epiblema strenuana* Walker has resulted in a significant reduction of *P. hysterophorus* vegetative growth in south-central Queensland (Shabbir et al., 2015) while a leaf-feeding beetle (*Zygogramma bicolorata* Pallister) was introduced in South Africa after rigorous research, which had demonstrated no significant risk to indigenous and economically important flora (Strathie, 2015). This beetle species has further recently been introduced in other African countries such as Ethiopia and

Tanzania but in the latter, little is known about the effectiveness of this bio-agent in controlling *P. hysterophorus***.**

Zygogramma bicolorata was first introduced in Tanzania from South Africa in March 2017. In the rainy season (January to March) 2018, *Z. bicolorata* was released into agricultural lands of Arusha and it became abundant within one year of introduction, resulting in a significant reduction in *P. hysterophorus* density in released areas (Ramadhan, pers. comm., April 2018).A study conducted by Ramadhan (pers. comm., April - October 2018) on the assessment of the presence of *Zygogramma bicolorata* in the release sites shows that bio agent is environmentally safe as it did not pose adverse effects to the environment and the crops in the release sites. During dry conditions, *Z. bicolorata* undergo diapause in the soil and emerged during the first rains in December where there were plenty of young *P. hysterophorus* plants. Adult *Z. bicolorata* lay eggs predominantly on *P. hysterophorus* leaves and their incubation period takes about six to seven days, after which larvae feed on the leaves (Chaudhari et al., 2012). The entire life cycle of the beetle takes about 40-42 days (Chaudhari et al., 2012). Newly hatched larvae initially feed on the terminal and axillary buds, which results in a reduction of the number of flowers, but as they grow, they consume the leaves of *P. hysterophorus* (Manjunath, 2010).

We aimed to quantify the effectiveness of *Z. bicolorata* as bio-agent in reducing *P. hysterophorus* vegetative growth and documenting the number of eggs, larvae, and adults produced over 56 days of the experiment under different controlled beetle treatments and different seasons (wet and dry). We further aimed to study the population dynamics of different life stages of the bio-control agent *Z. bicolorata.* We used different population sizes of beetles and expected that our large populations of *Z. bicolorata* (30 beetles) would suppress the vegetative growth stage of *P. hysterophorus*.

Material and methods

Study area

The effectiveness of *Z. bicolorata* as a bio-agent for the control of *P. hysterophorus* was evaluated under cage conditions at the Tropical Pesticides Research Institute (TPRI) in Arusha, Tanzania (3^{o}19' 51.35" S, 36^{o}37' 37.602" E) from February - July 2019 (wet) and August 2019 – January 2020 (dry).

Parthenium hysterophorus seed collection and nursery establishment

About 30,000 seeds of *P. hysterophorus* were collected in the field from individual matured plants that grew at least 5 m away from each other at Burka and Ngaramtoni in northern Tanzania during January 2019 and August 2019. The seeds were washed with distilled water, disinfected with 4% NaOCl for 10 minutes, air-dried for four days, and then germinated in the TPRI nursery (Kigel, 2017). After 14 days of nursery establishment, *P. hysterophorus* seedlings were transplanted into the experimental plots and after additional 10 days of acclimatization (before flowering), beetles were released at equal ratios of males and females per plot (at 10, 20, 30 and 0 individuals as control).

Study setup

Experimental plots of 4 m^2 comprised five rows of *P. hysterophorus* plants each with a distance of 1 m between plots and 1.5m between replicates. Fourteen days old seedlings of *P. hysterophorus* were transplanted to a quadrat of 25 cm x 25 cm, making a total of 25 plants per plot. The experimental plots were arranged in a Complete Randomized Design (CRD) with four treatments. Four treatments organized into Treatment 1 = No beetle, Treatment 2 = 10 beetles, Treatment 3 = 20 beetles, and Treatment 4 = 30 beetles replicated three times. White mesh nets were used to cover each plot and prevent the beetles from escaping.

Zygogramma bicolorata collection and release

Adult *Z. bicolorata* were collected from the field on *P. hysterophorus* plants at Burka ward, about 18 km away from TPRI, where they had been released in a test trial by TPRI in 2018. The beetles were collected in April, during the rainy season, when *Z. bicolorata* were emerging from the soil after diapause. They were then transported to the TPRI laboratory and their mated pairs were identified following the methods by Singh et al. (2017). Three plastic beakers with the same number of male and female beetles were introduced to 24 days old *P. hysterophorus* plants in the cages. Sets of **t**en plants each were selected randomly on each plot and tagged. Then the number of leaves eaten and the number of flowers per set of ten plants was recorded at 14 - days intervals while the plant heights and biomasses were recorded at the end of the experiment **(**after 56 days). Similarly, the number of beetle **e**ggs, larvae, and adults were collected after every fourteen days in the morning from each treatment.

Data analyses

Data were first tested for normality and homogeneity of variance using Shapiro-Wilk and Levine's test (Shapiro & Wilk, 1965). Analysis of variance (One-way ANOVA) was performed and means of different treatments were compared using Tukey's HSD. Treatments (number of beetles) were considered as independent variables while the number of flowers, leaves eaten by beetles, plant height, biomass, beetle eggs, larvae, and adults were considered as dependent variables were analyzed using STATISTICA version 8 Stat Soft Inc. (2007) at 5% level of significance. Two-factorial ANOVA was used to test the factors and interaction between season and treatment for different response parameters, tested separately over time, i.e., experimental days. Origin (2013) version 9.0 SR1 was used for plotting.

Results

Effects of Z. bicolorata on P. hysterophorus growth

The level of defoliation of *P. hysterophorus* (the number of leaves eaten) in the wet season increased with time and the effects were seen in all treatments of *Z. bicolorata* (Figure 1). The percentage of *P. hysterophorus* leaves eaten by *Z. bicolorata* increased rapidly from 14 to 28 days by 115%, 112% and 100% at the population of 10, 20 and 30, respectively, but no leaves were damaged in the control treatment (0 beetles) (Figure 1). There was a significant difference across treatments at 14 and 28 days after the *Z. bicolorata* release ($F_{3,8} = 58.81$, $P = 0.001$ and $F_{3,8} = 39.34$, $P = 0.001$, *respectively*). In the subsequent periods (28 to 42 days after beetle release), the percentage of leaves eaten increased by 107%, 83% and 75% for the populations of 10, 20 and 30 beetles, respectively, differing significantly across treatments at 42 days after the *Z. bicolorata* release ($F_{3,8} = 98.64$, $P = 0.001$). *Similarly, from day 42 to 56, there was a slight increase of 62%, 26% and 3%* corresponding to the population of 10, 20 and 30 beetles, respectively, while there was a significant difference across treatments at 56 days after *Z. bicolorata* release ($F_{3,8} = 57.23$, $P = 0.001$; *Figure 1).*

Our dry season experiment showed similar trends (Figure 1). The percentage of *P. hysterophorus* leaves eaten by *Z. bicolorata* increased rapidly from 14 to 28 days by 214%, 134% and 90% at the population of 10, 20 and 30, respectively, but no leaves were damaged in the control treatment (0 beetles) (Figure 1). There was a significant difference across treatments at 14 and 28 days after the *Z. bicolorata* release ($F_{3,8} = 1396.20$, $P = 0.001$ and $F_{3,8} = 42.60$, $P = 0.001$, *respectively*). In the subsequent periods (28 to 42 days after beetle release), the percentage of leaves eaten increased by 745%, 599% and 546% for the populations of 10, 20 and 30 beetles, respectively, differing significantly across treatments at 42 days after the *Z. bicolorata* release ($F_{3,8} = 92.71$, $P = 0.001$), *with only slight increases thereafter* ($F_{3,8} = 82.94$, $P = 0.001$; *Figure 1). In the full model, treatment significantly influenced response variables as did treatment x season while responses over the season were not significantly different (Table 3). When Z. bicolorata* was applied in the early days (14 to 28 days) of *P. hysterophorus* development in the wet season, we observed an initial increase in the number of flowers by 386%, 194%, 109% and 102% for the beetle population treatments of 0, 10, 20, and 30 individuals, respectively. The highest number of flowers was recorded after 28 days of the experiment and there was a significant difference across treatments ($F_{3,8} = 36.1, P = 0.001$). In the latter days (42 days after beetle release), the number of flowers was significantly lower than that of 28 days by 7%, 17%, 39% and 41% for the beetle population of 0, 10, 20, and 30 individuals respectively ($F_{3,8} = 57.1, P = 0.001$). *From day 42 to 56, the number of flowers reduced even further by 28%, 41%, 62% and 88%* for the beetle populations of 0,10, 20 and 30 individuals, respectively, which was significant across treatments at 56 days after *Z. bicolorata* release **(**$F_{3,8} = 35.92, P = 0.001$, *Figure 2*).

The *P. hysterophorus* plant heights significantly reduced by 47%, 72%, and 87% under *Z. bicolorata* populations of 10, 20, and 30 individuals, respectively, but not in the control **(**$F_{3,8} = 164.70, P = 0.001$, Figure. 3a). Similarly, the fresh above-ground biomass of *P. hysterophorus* subjected to different *Z. bicolorata* treatments was significantly and progressively reduced with increasing individuals of *Z. bicolorata.* The *Parthenium hysterophorus* biomass was significantly reduced by 44%, 73%, and 91% under *Z. bicolorata* populations of 10, 20, and 30 individuals, respectively, but not in the control treatment ($F_{3,8} = 100.52, P = 0.001$, Figure. 3b).

In the dry season, the number of flowers increased by 388%, 196%, 174% and 126% in the early days (14 to 28 days) of *P. hysterophorus* development for the beetle population treatments of 0, 10, 20, and 30 individuals, respectively, and there was a significant difference across treatments at 14 to 28 days ($F_{3,8} = 34.64, P = 0.001$ and $F_{3,8} = 32.67, P = 0.001$).In the latter days (42 days after beetle release), the number of flowers increased significantly from 28 days by 20%, 14%, 9% and 3% for the beetle population of 0, 10, 20, and 30 individuals, respectively

($F_{3,8}$ = *25.08, P = 0.001*). *From day 42 to 56, the number of flowers reduced even further by 32%, 44%, 71% and 92%* for the beetle populations of 0,10, 20 and 30 individuals, respectively, which was significant across treatments at 56 days after *Z. bicolorata* release **(**$F_{3,8}$ *= 27.81, P = 0.001, Figure 2*). *In the full model, treatment significantly influenced response variables as did treatment x season while responses over the season were not significantly different (Table 3)*

The *P. hysterophorus* plant heights were significantly reduced by 51%, 77%, and 90% under *Z. bicolorata* populations of 10, 20, and 30 individuals compared to the control **(**$F_{3,8}$ = *1114.66, P = 0.001,* Figure. 3c). Similarly, the fresh above-ground biomass of *P. hysterophorus* subjected to different *Z. bicolorata* treatments was significantly and progressively reduced with increasing individuals of *Z. bicolorata.* The *Parthenium hysterophorus* biomass was significantly reduced by 69%, 72%, and 87% under *Z. bicolorata* populations of 10, 20, and 30 individuals, respectively, compared to the control treatment **(**$F_{3,8}$ = *131.75, P = 0.001*, Figure. 3d. *In the full model, treatment significantly influenced response variables as did treatment x season while responses over the season were not significantly different (Table 3).*

Number of Z. bicolorata eggs, larvae and adults over time

In the wet season experiment, the number of eggs and released adults differed significantly across treatments 14 days after beetle introduction (eggs: $F_{3,8}$ = *95.11, P < 0.001*; adults: $F_{3,8}$ *= 481.71, P < 0.001, Tables 1*) but not the number of larvae ($F_{3,8}$ = *2.32, P = 0.137*). The highest number of eggs was recorded at the highest treatment density (30 beetle individuals) in the first 14 days. In the latter days (42 days after beetle release), the treatment with the highest beetle population (30 beetle individuals) resulted in the highest number of larvae ($F_{3,8}$ = *740.21, P < 0.001*) while the highest number of adults were recorded after 56 days for the same beetle population treatment **(**$F_{3,8}$ = *41.92, P < 0.001,* Table 2).

On the other hand, in the dry season, fourteen days after beetle introduction, the number of eggs, larvae and released adults differed significantly across treatments **(**$F_{3,8} = 72.32$, $P < 0.001$; $F_{3,8} = 104.87$, $P < 0.001$, *and* $F_{3,8} = 99.60$, $P < 0.001$, *respectively, Table 2*). The highest number of eggs was recorded at the highest treatment density (30 beetle individuals) after 28 days and there was a significant difference across treatments ($F_{3,8} = 95.11$, $P < 0.001$, Table 2)**.** In the latter days (42 days after beetle release), the treatment with the highest beetle population (30 beetle individuals) resulted in the highest number of larvae **(**$F_{3,8} = 137.92$, $P < 0.001$) while the highest number of adults were recorded after 56 days for the same beetle population **(**$F_{3,8} = 117.86$, $P < 0.001$, Table 2).

Discussion

We found that the defoliation caused by the biocontrol agent *Z. bicolorata* had an immediate adverse effect upon *P. hysterophorus* performance. This study has shown that *Z. bicolorata* was effective in reducing the number of *P. hysterophorus* leaves and flowers already at low beetle populations (10 beetles) and even further when the population increased to 30 beetles both in the wet and dry season. However, in the early days (14 – 28) number of leaves eaten during the dry season were few compared to wet and this might be associated with weather condition whereby this period had a long dry season which limited *Z. bicolorata* population build-up. Similar results were reported by Dhileepan et al. (2000), who found complete defoliation of *P. hysterophorus* by *Z. bicolorata* under water stress compared to the areas without water. In addition, there was only a slight increase in the number of *P. hysterophorus* leaves eaten for the highest beetle population at the later stage of our experiment, i.e., from 42 to 56 days, which highlights that with depletion of leaves also the foraging activity slows down.

Furthermore, the number of flowers produced during the dry season was slightly lower compared to those produced during the wet season, this might highlight that with an increase in prolonged dry periods, the number of flower production gets scarce (Rivera and Borchert, 2001). Similarly, *P. hysterophorus* produced more flowers in the latter days (42 days) in the dry than in the wet season, when flowers were produced in the early days (28 days). This might highlight that soil moisture might be a limiting factor delaying flower production. Although *Z. bicolorata* does not feed on *P. hysterophorus* flowers, a reduction in the number of flowers is associated with the feeding behavior of larvae and adults, which tend to congregate and feed on tender leaves, terminal and axillary buds and, thereby, lead to stunted growth and fewer flowers (Manjunath, 2010; Mcconnachie, 2015). Similarly, our findings agree with *Z. bicolorata* studies in India, where *P. hysterophorus* plants were suppressed and their seed production was reduced by 98% (Jayanth and Bali, 1994). In central, southern, and northern Queensland, a total of 400,000 adults *Z. bicolorata* were released to control *P. hysterophorus* (Dhileepan and Strathie, 2009), which is similar to our study.

We further found that *P. hysterophorus* height and biomass were highly reduced, probably due to stress caused by the beetles (Wang et al., 2015). This is because *P. hysterophorus* was heavily skeletonized by *Z. bicolorata* which probably resulted in to decrease in height and above-ground fresh biomass. Plants with few leaves are poor competitors for light and are more susceptible to petal and sepal abscission, which results in poor nectar availability and, hence, low seed dispersal (Saifuddin et al., 2010) as well as reduced biomass (Falster and Westoby, 2003). Shabbir et al. (2016) also reported that shoot height and dry biomass were significantly compromised after the introduction of high *Z. bicolorata* densities. Biological studies carried out with *Z. bicolorata* in Bangalore revealed that adult *Z. bicolorata* individuals are capable of undergoing diapause, burrow in the soil at about 3 cm depth for more than six months in the absence of *P. hysterophorus* and emerge with the onset of monsoon (Jayanth and Bali, 1995). They reported that soil moisture plays an important role in and adult emergence of *Z. bicolorata* while long dry spells can significantly reduce *Z. bicolorata* populations under field conditions. Adkins and Shabbir (2014) also recommended *Z. bicolorata* as an environmentally friendly biocontrol method, which does not impact non-targeted species and seems to be sustainable in *P. hysterophorus* control.

Zygogramma bicolorata eggs, larvae, and adults varied particularly in the timing of production across the season. The highest numbers of eggs were laid after 28 days in the dry period while in the wet season, these were recorded after 14 days only. This variation might be associated with the moisture condition in the soil as *Z. Bicolorata* requires high moisture content to lay eggs and complete its life cycle (Singh et al., 2017). Moreover, in this study across both seasons, beetles released in large population sizes were capable of almost completely defoliating the 2 x 2 m plot (i.e., 25 individual plants) within 42 up to 56 days. Singh et al. (2017) confirmed that both larvae and adult *Z. bicolorata* feed on the leaves of *P. hysterophorus* and that the beetles complete their life cycles exclusively in *P. hysterophorus* plants. Different studies have recommended the use of biocontrol agent *Z. bicolorata* as an appropriate agent for controlling *P. hysterophorus* in Nepal (Shrestha et al., 2011), India (Jayanth and Bali, 1993; Manjunath, 2010; Chaudhari et al., 2012), Southern Asia (Dhileepan and Senaratne, 2009), Australia (Dhileepan, 2001) and in South Africa (Strathie, 2015). Similarly, this study has highlighted the suppressive effect of *Z. bicolorata* on *P. hysterophorus,* confirming that it can be widely practiced in Tanzania as well.

We recommend that beetles should be introduced at densities of at least five male and female individuals per m^2 for high effectiveness. Furthermore, (Smith and De Bach, 1942) highlighted criteria that help in measuring the success biocontrol programme, which are a) reduction in the number of pest b) pest population maintenance at low level after natural enemy establishment, and c) a high rate of survival of the pest populations when protected from their natural enemies. We showed in our controlled experiments that *Z. bicolorata* meets the first and third criteria as a biocontrol agent against *P. hysterophorus* (Smith and De Bach, 1942) since the beetles reduced plant height, damaged leaves, and reduced the number of flowers, i.e., the potential future generation. Our study also confirmed that *Z. bicolorata* was capable of defoliating *P. hysterophorus* within forty-two to fifty-six days particularly when it was released at high population densities (30 beetles).

We conducted the same experiment across two different seasons to assess the performance of beetles under different environmental conditions, particularly soil moisture. The overall performance of the beetle was similar across the seasons but timings were slightly different. The number of leaves eaten during the dry season was higher in the latter days of the experiment, indicating that beetle release should preferably be conducted during the wet season when beetle populations grow more quickly and can reduce *P. hysterophorus* more effectively.

While highlighting the importance of the beetle as bio-agent against *P. hysterophorus*, we are also aware that combined effects of *Z. bicolorata* and other methods such as competitive native plants and using plant extracts might be needed for long-term control of the invasive. For example**,** Shabbir et al. (2015) have shown that competitive pasture plant species such as the butterfly pea (*Clitoria ternatea L*) and buffel grass (*Cenchrus ciliaris L*) together with a biological control agent (*Epiblema strenuana* Walker) can synergistically act to reduce *P. hysterophorus* biomass up to 69% in southern central Queensland. Hence, these species might be additional potential bio-agents that could be used in Tanzania in combination with the beetle to suppress *P. hysterophorus* in the long run. Moreover, to confirm the safe use of the beetle after release in the wild in Tanzania, we recommend proper field studies that verify whether the beetles do not feed on other plants in the wild once *P. hysterophorus* is depleted. In conclusion, our study shows that *P. hysterophorus* can be controlled effectively by using *Z. bicolorata*, particularly at high population size, and when it is applied before the flowering stage of the invasive. More importantly, local communities should be trained on the identification of male and female *Z. bicolorata* to ensure an increase in the beetle population over time.

Acknowledgments

The authors would like to thank Rufford Small Grant for nature conservation, grant number 27383-1 and the Centre for Research, Agricultural advancement, Teaching Excellence and Sustainability (CREATES, supported by the World Bank) for supporting this study. Thanks go to D. Samwely and M. Macha for help in collecting *P. hysterophorus* seeds, transplanting of seedlings, and data collection in the experimental site. The authors also thank the Tropical Pesticides Research Institute (TPRI) for granting permission to conduct these experiments.

Conflict of Interest: Authors declare there is no conflict of interest.

REFERENCES

Adkins, S., and A. Shabbir. 2014. Biology, ecology, and management of the invasive parthenium weed (Parthenium hysterophorus L.). *Pest Management Science*, *70*(7), 1023-1029. doi:https://doi.org/10.1002/ps.3708

Adkins, S. W., A. Shabbir, and K. Dhileepan. 2018a. *Parthenium weed: biology, ecology, and management* (Vol. 7): CABI.

Adkins, S. W., A. Shabbir, and K. Dhileepan. 2018b. *Parthenium Weed: Biology, Ecology, and Management* (Vol. 11): CABI.

Ayele, S., L. Nigatu, T. Tana, and S. W. Adkins. 2013. Impact of parthenium weed (Parthenium hysterophorus L.) on the above-ground and soil seed bank communities of rangelands in Southeast Ethiopia. *International Research Journal of Agricultural Science and Soil Science, 3*(7), 262-274.

Chaudhari, S. V., P. L. Gade, V. S. Tanpure, and P. S. Chaudhari. 2012. Ecofriendly Control of Carrot Weed Parthenium hysterophorus using Mexican Beetle Zygogramma bicolorata around Rahuri City (Maharashtra-India). *International Journal of Innovative Research and Development (ISSN 2278–0211), 1*(4), 238-245.

Cock, M., and J. Holloway. 1982. The history of, and prospects for, the biological control of Chromolaena odorata (Compositae) by Pareuchaetes pseudoinsulata Rego Barros and allies (Lepidoptera: Arctiidae). *Bulletin of Entomological Research, 72*(2), 193-205.

Dhileepan, K. 2001. Effectiveness of introduced biocontrol insects on the weed Parthenium hysterophorus (Asteraceae) in Australia. *Bulletin of entomological research, 91*(3), 167-176.

Dhileepan, K., S. Setter, and R. Mcfadyen. 2000. The response of the weed Parthenium hysterophorus (Asteraceae) to defoliation by the introduced biocontrol agent Zygogramma bicolorata (Coleoptera: Chrysomelidae). *Biological Control, 19*(1), 9-16.

Dhileepan, K., and L. Strathie. 2009. Parthenium hysterophorus L.(Asteraceae). *Biological control of tropical weeds using arthropods*, 274-318.

Dhileepan, K., and K. Senaratne. 2009. How widespread is Parthenium hysterophorus and it's biological control agent Zygogramma bicolorata in South Asia? *Weed Research, 49*(6), 557-562.

Dogra, K. S., S. K. Sood, and R. Sharma. 2011. Distribution, biology, and ecology of Parthenium hysterophorus L.(congress grass) an invasive species in the North-Western Indian Himalaya (Himachal Pradesh). *African Journal of Plant Science*, *5*(11), 682-687.

Falster, D. S., and M. Westoby. 2003. Plant height and evolutionary games. *Trends in Ecology and Evolution, 18*(7), 337-343. doi:https://doi.org/10.1016/S0169-5347(03)00061-2

Jayanth, K., and G. Bali. 1993. Biological studies on Zygogramma bicolorata Pallister (Coleoptera: Chrysomelidae), a potential biocontrol agent of Parthenium hysterophorus L.(Asteraceae). *Journal of Biological Control., 7*(2), 93-98.

Jayanth, K., and G. Bali. 1994. Biological control of Parthenium hysterophorus by the beetle Zygogramma bicolorata in India. *Boletin Fitosanitario 42*(4), 207-213.

Jayanth, K., and G. Bali. 1995. Effect of soil moisture on pupation and adult emergence of Zygogramma bicolorata Pallister (Coleoptera: Chrysomelidae). *Journal of Entomological Research, 19*(2), 183-185.

Kigel, J. 2017. Seed germination in arid and semiarid regions. In *Seed development and germination* (pp. 645-699): Routledge.

Kija, B., C. N. Mweya, M. Mwita, and R. Fumagwa. 2013. Prediction of suitable habitat for potential invasive plant species Parthenium hysterophorus in Tanzania: a short communication. *International Journal of Ecosystem, 3*(4), 82-86. doi: https://doi.org/10.5923/j.ije.20130304.04

Kilewa, R., and A. Rashid. 2014. Distribution of invasive weed Parthenium hysterophorus in natural and agro-ecosystems in Arusha Tanzania. *International Journal of Science and Research, 3*(12), 1-4.

Kumar, S. 2009. Biological control of Parthenium in India: status and prospects. *Indian Journal of Weed Science, 41*(1-2), 1-18.

Manjunath, T. (2010). *Biological control of the wasteland weed, Parthenium hysterophorus, in India–An overview.* Paper presented at the Presented at the 3rd International Conference on Parthenium organized by and at IARI.

Mcconnachie, A., L. Strathie, W. Mersie, L. Gebrehiwot, K. Zewdie, A. Abdurehim, B. Abrha, T. Araya, F. Asaregew, and F. Assefa. 2011. Current and potential geographical distribution of the invasive plant Parthenium hysterophorus (Asteraceae) in eastern and southern Africa. *Weed Research, 51*(1), 71-84.

Mcconnachie, A. J. 2015. Host range and risk assessment of Zygogramma bicolorata, a defoliating agent released in South Africa for the biological control of Parthenium hysterophorus. *Biocontrol Science and Technology, 25*(9), 975-991.

Navie, S., R. Mcfadyen, F. Panetta, and S. Adkins. 1996. The biology of Australian weeds. 27. Parthenium hysterophorus L. *Plant Protection Quarterly, 11*(2), 76-88.

Ngondya, I. B., L. K. Munishi, A. C. Treydte and P. A. Ndakidemi. 2016. A nature-based approach for managing the invasive weed species Gutenbergia cordifolia for sustainable rangeland management. *SpringerPlus, 5*(1), 1787. doi:https://doi.org/10.1186/s40064-016-3480-y

Rivera, G., and R. Borchert. 2001. Induction of flowering in tropical trees by a 30-min reduction in photoperiod: evidence from field observations and herbarium specimens. *Tree Physiology, 21*(4), 201-212.

Saifuddin, M., A. Hossain, and O. Normaniza. 2010. Impacts of shading on flower formation and longevity, leaf chlorophyll, and growth of Bougainvillea glabra. *Asian Journal of Plant Sciences, 9*(1), 20.

Seta, T., A. Assefa, F. Mesfin, and A. Balcha. 2013. Distribution status and the impact of parthenium weed (Parthenium hysterophorus L.) at Gedeo Zone (Southern Ethiopia). *African Journal of Agricultural Research, 8*(4), 386-397. doi:https://doi.org/10.5897/AJAR12.1646

Shabbir, A., K. Dhileepan, M. P. Zalucki, C. O'Donnell, N. Khan, Z. Hanif and S. W. Adkins. 2015. The combined effect of biological control with plant competition on the management of parthenium weed (Parthenium hysterophorus L.). *Pakistan Journal of Botany, 47*(SI), 157-159.

Shabbir, A., S. Sadia, and I. Mujahid. 2016. Biocontrol efficiency of Zygogramma bicolorata at different growth stages of Parthenium hysterophorus. *Indian Journal of Weed Science, 48*(4), 460-463.

Shrestha, B., A. Shabbir, and S. Adkins. 2015. Parthenium hysterophorus in Nepal: a review of its weed status and possibilities for management. *Weed Research, 55*(2), 132-144. doi:https://doi.org/10.1111/wre.12133

Shrestha, B. B., K. B. Thapa-Magar, A. Paudel, and U. B. Shrestha. 2011. Beetle on the battle: Defoliation of Parthenium hysterophorus by Zygogramma bicolorata in Kathmandu valley, Nepal. *Botanica Orientalis: Journal of Plant Science, 8*, 100-104.

Singh, S., P. K. Gill, H. Dhaliwal, and V. Kumar. 2017. Life cycle and effectiveness of Zygogramma bicolorata Pallister (Chrysomelidae: Coleoptera) on Parthenium hysterophorus eradication. *Journal of Global Agriculture and Ecology, 7*(2), 60-65.

Smith, H. S., and P. De Bach. 1942. The measurement of the effect of entomophagous insects on population densities of their hosts. *Journal of Economic Entomology, 35*(6), 845-849.

Strathie, L. 2015. Managing the invasive alien plant Parthenium hysterophorus in South Africa. *Grassroots, 15*(2), 15-21.

Wabuyele, E., A. Lusweti, J. Bisikwa, G. Kyenune, K. Clark, W. D. Lotter, A. J. Mcconnachie and M. Wondi. 2014. A roadside survey of the invasive weed Parthenium hysterophorus (Asteraceae) in East Africa. *Journal of East African Natural History, 103*(1), 49-58. doi:https://doi.org//10.2982/028.103.0105

Wang, D., Y. Liu, X. Tan, H. Liu, G. Zeng, X. Hu, H. Jian, and Y. Gu. 2015. Effect of exogenous nitric oxide on the antioxidative system and S-nitrosylation in leaves of Boehmeria nivea (L.) Gaud under cadmium stress. *Environmental Science and Pollution Research, 22*(5), 3489-3497.

List of figures

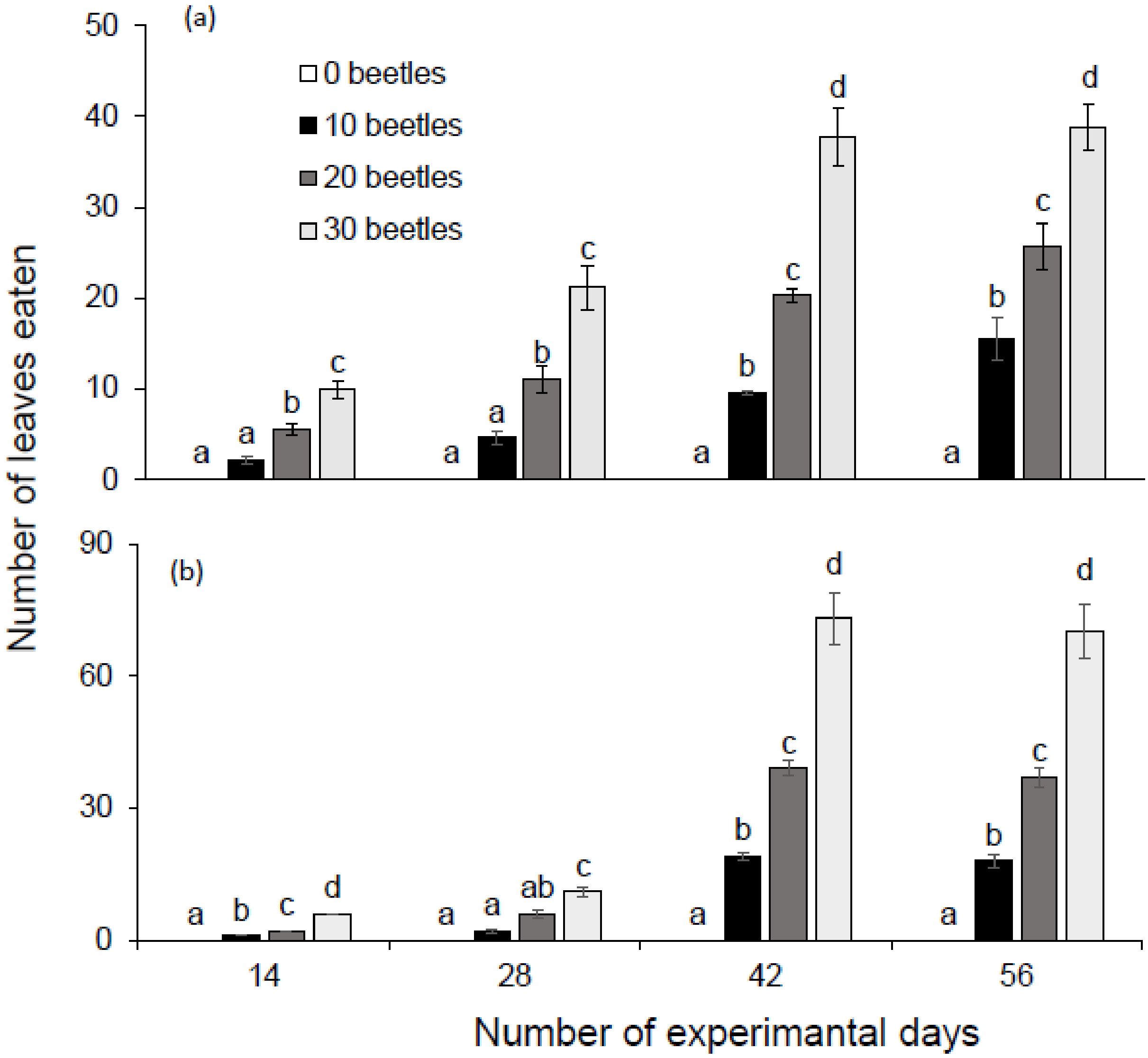

Figure 1. Average ± SE number of *Parthenium hysterophorus* leaves eaten from (a) February – July 2019 (wet season) and (b) August 2019 – January 2020 (dry season) at 14, 28, 42 and 56 days after beetle release under caged conditions. Treatments were the release of 0, 10, 20 and 30 *Zygogramma bicolorata* individuals. Different letters indicate significant differences across treatments according to Tukey's HSD at $P < 0.05$.

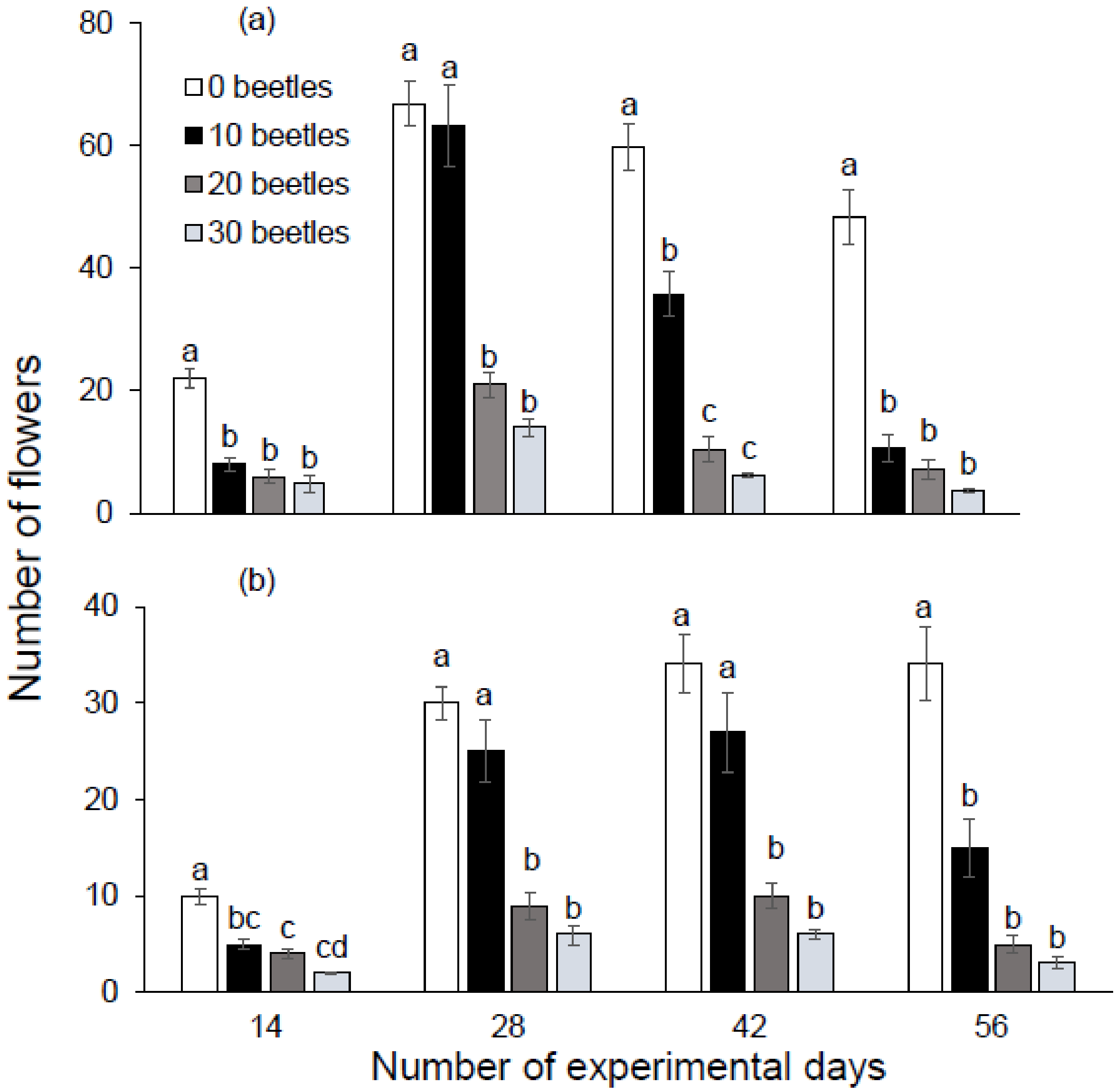

Figure 2. Average ±SE number of flowers produced by *Parthenium hysterophorus* from (a) February – July 2019 (wet season) and (b) August 2019 – January 2020 (dry season) at 14, 28, 42 and 56 days after *Zygogramma bicolorata* beetles were released at a density of 0, 10, 20 and 30 individuals under caged conditions. Different letters indicate significant differences across treatments according to Tukey's HSD at $P < 0.05$.

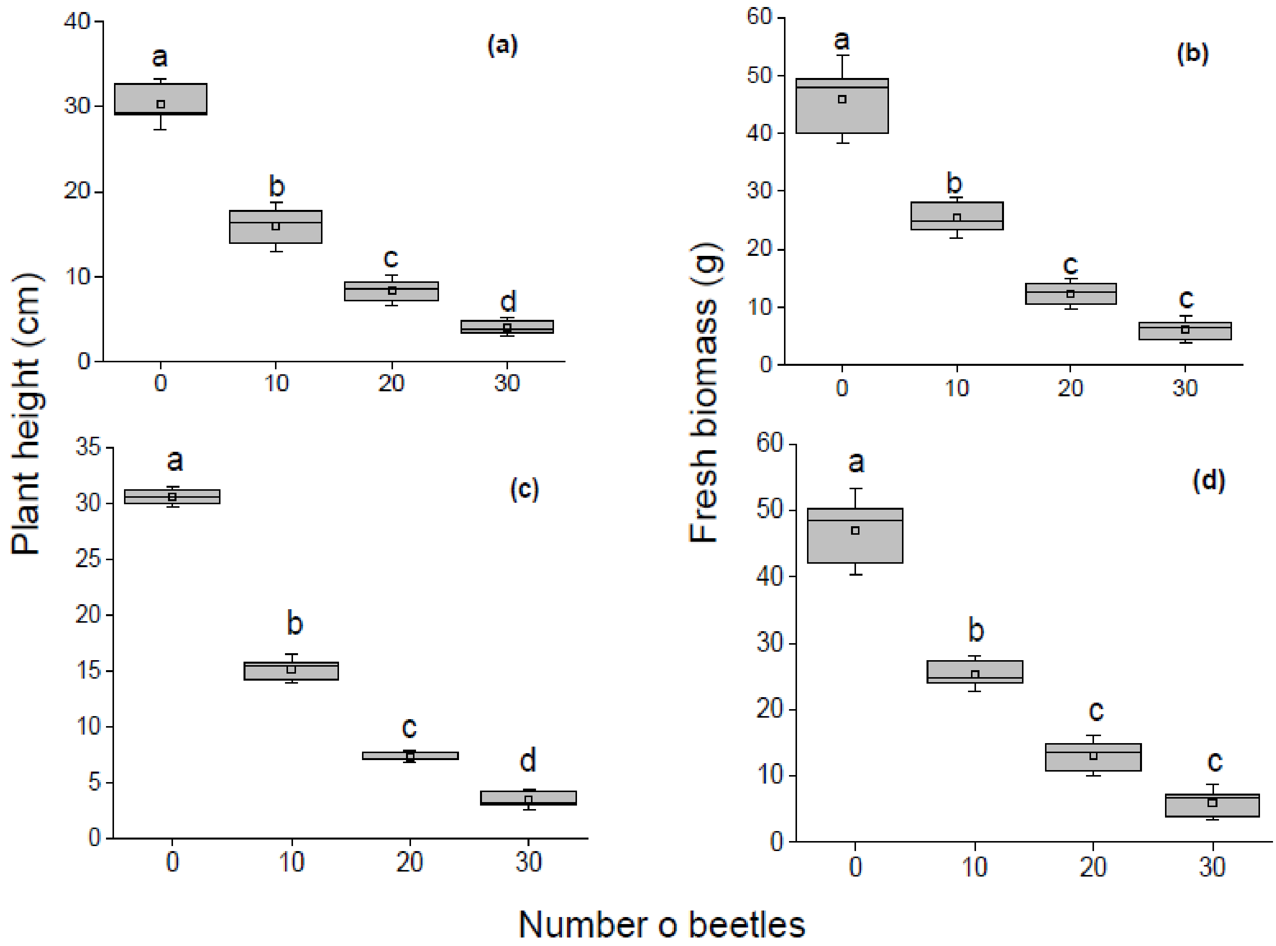

Figure 3. Box plots representing median (horizontal bar), quartiles (boxes) and Standard deviation (whiskers) of *Parthenium hysterophorus* (a) height and (b) fresh biomass from February – July 2019 (wet season) and (c) height and (d) fresh biomass from August 2019 – January 2020 (dry season) after being treated with *Zygogramma bicolorata* beetles under different treatments **(**0, 10, 20 and 30 individuals**)** for 56 days. Different letters indicate significant differences according to Turkeys HSD at $P < 0.05$.

Supplemental materials

Table 1. One-way ANOVA results comparing the average number of (a) eggs and (b) larvae produced and (c) adult beetles from February – July 2019 after *Zygogramma bicolorata* release at 14, 28, 42 and 56 days under screen house condition into *Parthenium hysterophorus* plant stands at the Tropical Pesticide Research Institute. Different letters across treatments (# beetles) indicate significant differences according to Tukey's HSD at $P < 0.05$.

(a)	**Treatment (# beetles)**						
	0	**10**	**20**	**30**	$F_{(3,8)}$	**P**	**% CV**
14 days	0^{a}	101^{b}	133^{c}	159^{d}	95.11	< 0.001	8.6
28 days	0^{a}	20^{b}	43^{c}	50^{c}	57.46	< 0.001	14.7
42 days	0^{a}	10^{b}	27^{c}	39^{d}	732.51	< 0.001	4.6
56 days	0^{a}	6^{a}	17^{b}	21^{b}	17.80	< 0.001	21.9
(b)	**Treatment (# beetles)**						
	0	**10**	**20**	**30**	$F_{(3,8)}$	**P**	**%CV**
14 days	0^{a}	5^{a}	8^{a}	22^{a}	2.32	0.137	111.2
28 days	0^{a}	5^{a}	15^{b}	28^{c}	146.80	< 0.001	15.7
42 days	0^{a}	9^{b}	25^{c}	33^{d}	740.21	< 0.001	5.5
56 days	0^{a}	11^{ab}	16^{b}	21^{b}	9.16	0.013	44.5
(c)	**Treatment (# beetles)**						
	0	**10**	**20**	**30**	$F_{(3,8)}$	**P**	**%CV**
14 days	0^{a}	3^{b}	13^{c}	21^{d}	481.71	< 0.001	7.0
28 days	0^{a}	5^{a}	15^{b}	28^{c}	146.82	< 0.001	15.0
42 days	0^{a}	10^{b}	161^{c}	237^{d}	1140.51	< 0.001	4.6
56 days	0^{a}	115^{b}	193^{c}	270^{d}	41.92	< 0.001	16.6

Table 2. One-way ANOVA results comparing the average number of (a) eggs and (b) larvae produced and (c) adult beetles from August 2019 – January 2020 after *Zygogramma bicolorata* release at 14, 28, 42 and 56 days under screen house condition into *Parthenium hysterophorus* plant stands at the Tropical Pesticide Research Institute. Different letters across treatments (# beetles) indicate significant differences according to Tukey's HSD at $P < 0.05$.

(a)	**Treatment (# beetles)**						
	0	**10**	**20**	**30**	$F_{(3,8)}$	**P**	**% CV**
14 days	0^{a}	10^{b}	22^{c}	25^{c}	72.32	< 0.001	13.5
28 days	0^{a}	51^{b}	67^{c}	80^{d}	95.11	< 0.001	8.6
42 days	0^{a}	38^{b}	57^{c}	73^{d}	121.68	< 0.001	7.6
56 days	0^{a}	10^{a}	25^{b}	37^{b}	202.04	< 0.001	8.1

(b)	**Treatment (# beetles)**						
	0	**10**	**20**	**30**	$F_{(3,8)}$	**P**	**%CV**
14 days	0^{a}	0.9^{b}	2.8^{c}	4.3^{d}	104.87	< 0.001	14.9
28 days	0^{a}	50^{b}	65^{c}	91^{d}	574.97	< 0.001	6.1
42 days	0^{a}	101^{b}	132^{c}	190^{d}	137.92	< 0.001	10.3
56 days	0^{a}	10^{b}	19^{c}	27^{d}	120.61	< 0.001	10.8

(c)	**Treatment (# beetles)**						
	0	**10**	**20**	**30**	$F_{(3,8)}$	**P**	**%CV**
14 days	0^{a}	1^{a}	7^{b}	12^{c}	99.60	< 0.001	21.7
28 days	0^{a}	2^{a}	7^{b}	14^{c}	92.00	< 0.001	10.4
42 days	0^{a}	63^{b}	87^{c}	128^{d}	78.13	< 0.001	12.3
56 days	0^{a}	130^{b}	225^{c}	327^{d}	117.86	< 0.001	9.9

Table 3; Two- factorial ANOVA showing the different statistics for each model including treatment (number of beetles, i.e., 0, 10, 20, 30 individuals) and season (dry and wet season) as factors.

Parameters	**factors**	**F**	***P-value***
Leaves 14	Treatment	128.9	< 0.001
	Season	1.2	0.321
	Season x treatment	8.5	< 0.001
Leaves 28	Treatment	73.2	< 0.001
	Season	0.07	0.91
	Season x treatment	6.8	0.004
Leaves 42	Treatment	170.9	< 0.001
	Season	0.06	0.81
	Season x treatment	16.9	< 0.001
Leaves 56	Treatment	137.1	< 0.001
	Season	1.1	0.21
	Season x treatment	13.2	< 0.001
Flowers 14	Treatment	45.9	< 0.001
	Season	0.1	0.72
	Season x treatment	4.3	0.02
Flowers 28	Treatment	63.5	< 0.001
	Season	0.4	0.71
	Season x treatment	7.3	0.003
Flowers 42	Treatment	135.2	< 0.001
	Season	1.2	0.21
	Season x treatment	49.5	0.006
Flowers 56	Treatment	62.9	< 0.001
	Season	0.6	0.65
	Season x treatment	8.9	0.003
Height	Treatment	596.51	< 0.001
	Season	1.4	0.25
	Season x treatment	10.1	0.001
Biomass	Treatment	228.6	< 0.001
	Season	0.1	0.76
	Season x treatment	8.1	0.002

Poster presentation